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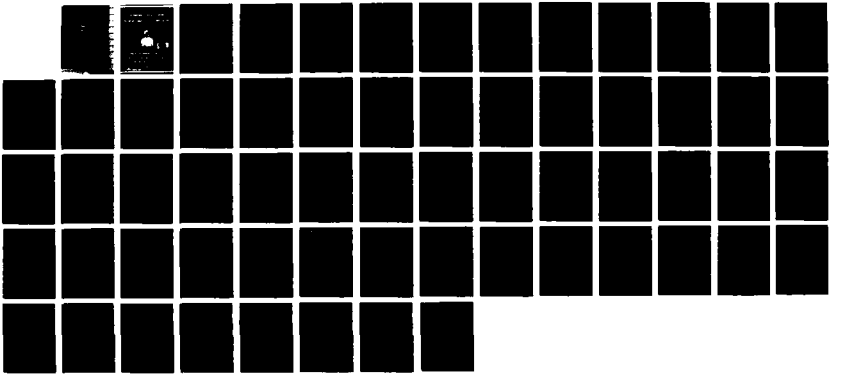

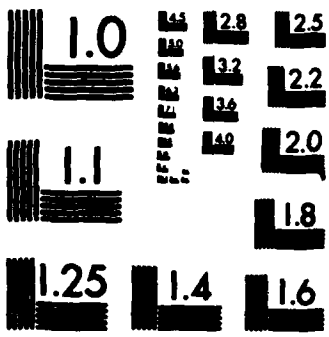

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ATROPINE'S EFFECTS UPON THE HEART AND ITS SYSTEMIC OUTPUT

DIVISION OF EXPERIMENTAL THERAPEUTICS

WALTER REED ARMY INSTITUTE OF RESEARCH

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Atropine affects the cardiovascular system in other ways besides the well-known acceleration of heart rate. However, knowledge of many of the other actions of atropine upon the cardiovascular system remains unclear or unknown. Much of this lack of knowledge can be traced to an insufficient understanding of the parasympathetic anatomical and physiological relationships within the heart, in general, and the ventricles, in particular. We have an incomplete knowledge of the effects of atropine upon the peripheral vasculature function and most particularly the venous system. This review traces the early historical development of the cardiovascular pharmacology of atropine. The influences of atropine upon the cardiac output, coronary circulation, myocardial energetics, work of the heart, exercise and the baroreceptors have been covered. A review of atropine's effects upon sweat production abatement and the related consequences to exercise performance in humans, horses and dogs appears. The anatomy and physiology of the heart's conduction system and the attendant autonomic influences have been discussed, including many of the theoretical and the unsolved aspects, such as, the

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the modulating theories of the parasympathetics upon the sympathetic innervation of the conduction system. The evidence for and against parasympathetic innervation of the ventricular myocardium has been presented. A summary of studies outlines the influence of human age upon the intensity of atropine's responses. I reviewed the evidence for atropine tachyphylaxis. Atropine promotes agonist as well as antagonist responses. A discussion of the effects of atropine upon the bradyarrhythmias following post myocardial infarcts appears, based mainly on clinical studies. An update and review of atropine chemistry includes a treatment of formulation stability and some of the sensitive assays presently available for tissue level determinations, which permits the current atropine pharmacodynamic studies to procede. In conclusion, we need further basic investigations into the physiology and pharmacology of muscarinic receptors and atropine formulations to advance our knowledge of atropine's actions on the cardiovascular system.

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ATROPINE'S EFFECT UPON THE HEART AND ITS SYSTEMIC OUTPUT

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Division of Experimental Therapeutics
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PREFACE

This review represents background literature studies undertaken to support a protocol and the active research in the author's laboratory. Although atropine represents an old drug commonly used as a medicament and a research tool, little information regarding its effects upon the heart and the cardiovascular system appears in a single source within the professional literature. Atropine has received considerable attention in recent times as an antidote for organophosphate poisoning and also as a possible remedy for the treatment of the bradyarrhythmias which can result from posterior myocardial infarcts. The author hopes that this manuscript will assist others who pursue answers to this drug's actions upon the heart and the systemic circulation.

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I wish to express my appreciation to all who make the National Library of Medicine a functional reality and make such an undertaking as this a much easier task than it might have been. Last, but not least, I do want to thank my wife, Martha, for putting up with my numerous nightly and weekend trips to the National Library of Medicine.

ATROPINE'S EFFECTS UPON THE HEART AND ITS SYSTEMIC OUTPUT

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I. INTRODUCTION

Ever since the 1940's, organophosphate compounds have posed a health hazard to civilian[388] and military populations.[392] Not only do some of these compounds serve as martial devices, but their extensive use in agriculture has caused accidental poisoning. Organophosphate compounds manifest their highly toxic responses by blocking cholinesterase function. Foremost in consideration for prophylactic and antagonist use has been atropine. Intramuscular atropine sulfate has become the initially prescribed treatment of choice should an overt military use appear imminent. As presently conceived, 2 mg of atropine sulfate in a self-injection module shall be issued to military personnel. Thus, individuals with little medical knowledge shall perform intramuscular self-injections. Such reliance on individual judgement and administration raises questions about the amount of atropine sulfate an individual can withstand, yet remain reasonably functional. Certainly great concern remains for atropine dosage after organophosphate poisoning, however, the consideration becomes one of insuring survival not one of mobility. In this review major emphasis centers upon the effects of atropine upon cardiac pacemaker, conduction and inotropy and changes noted in peripheral arterial resistance.[389] An overview of atropine's use in postmyocardial infarcts adds to our knowledge of this drug's action upon the conduction system. Lastly, I felt an update in chemistry appeared in order due to an almost total lack of attention being paid to atropine's stability in manufacture and use in injectable form.

II. HISTORICAL BACKGROUND

Earnest studies in the action of atropine followed Mein's isolation of pure atropine in 1831. Von Bezold and Bloebaum first described the vagolytic effect of atropine on the heart (dl-hyoscyamine) in 1867. Soon after, from experiments in the frog's heart, Schmeideberg described the action of atropine, as taking place after the post-ganglionic nerve endings.[75, 119] Studies by Anrep[10] demonstrated an initial reduction in heart rate following administration of .06 mg of atropine subcutaneously in the dog. Muller, in 1891, first described differences in elevated heart rate response to atropine exhibited by humans of various age groups. He noted that the greatest augmentation of heart rate in response to atropine occurred in persons about 25 years of age and that a rapid decline in heart rate response follows atropine after the age of 55.[237]

Although various pharmacological studies appeared in the literature following Muller's observations, a concerted effort did not appear until just after World War I. Possibly, impetus for additional studies on the actions of atropine stemmed from the paper of Marris.[228] Following intramuscular injection of 2 mg of atropine, an augmentation of heart rate occurred within 25 minutes in normal and most febrile patients; however, those patients with typhoid or paratyphoid A and B infections did not manifest an increase in heart rate. During this era epidemics of typhoid occurred frequently throughout the world.

Sturgis et al., in 1919,[351] demonstrated that a subcutaneous injection of atropine sulfate caused an initial drop in heart rate, in most cases, followed by a subsequent rise in heart rate. These authors found that diastolic aortic blood pressure rose, and that systolic aortic blood pressure elicited a more variable response from person-to-person, while the aortic pulse pressure declined in all individuals. The level of heart rate elevation appeared greatest in individuals with arrhythmias of undefined origin. The human subjects studied by Sturgis et al.[351] showed no statistical change in total body oxygen utilization at 3-5 minutes following atropine injection (during the period of reduced heart rate) or 17 minutes following injection (when heart rate augmented above control levels). Independent studies by McGuigan,[237] Harris[136] and Heinekamp[141] in the early 1920's substantiated the earlier findings of Anrep[10] and Sturgis and associates,[351] that small doses of atropine sulfate (less than 1 mg) usually caused a slowing of the heart. Harris[136] found that the blood pressure fell; further, this investigator reported a reduced heart rate that remained suppressed for at least 1 hour. Such a long duration of suppressed heart rate which Harris observed in humans, has not been confirmed. Heinekamp[141] demonstrated that the suppressed heart rate caused by atropine does not occur following sectioning of the vagi in the dog. Therefore, he proposed that atropine in small doses caused a central vagal stimulation at the

level of the dorsal motor nucleus of the medulla oblongata. Observations by McGuigan, Harris and Heinekamp indicated that the duration of heart rate reduction depends upon the strength and rate of atropine injected and on the route used; that is, a subcutaneous, intramuscular or intravenous injection. Two investigators[239, 115] working independently corroborated the earlier work of Miller whereby a heart rate response of atropine reaches a peak between 20-30 years of age, then diminishes with age to about half the response,[237] or to very little response past 50 years of age.[115] Crawford[52a] showed no discernible age difference in heart rate response after atropine due to gender. Using trained, unanesthetized dogs, Marshall[229] confirmed previously observed hemodynamics following the administration of 4 mg of atropine. These observations included a doubling of heart rate and the lack of total body oxygen consumption change. Marshall showed that stroke volume diminished by as much as 50–67%, yet cardiac output rose only slightly (110%) following atropine. Smith et al.[34] administered atropine sulfate to humans in the resting state and noted the same responses as Marshall observed in dogs the previous year. They found a severe depression in stroke volume (25% of control), which nearly returned to preinjection levels by one hour. At about the same time a study in humans[346] showed the same heart rate responses previously reported and further demonstrated that atropine administered intravenously did not affect the expected oxygen contents of arterial or mixed venous blood. In a study involving 62 healthy medical students,[274] administration of 2 mg of atropine sulfate subcutaneously caused an increase in heart rate (151 ± 3% SEM) in 61 of the students. For reasons unclear to the author one subject exhibited a 15 percent reduction in heart rate following the same treatment. The merits of this study demonstrate the consistency of response by a large sample population with the exception of one individual, to a fixed regimen of atropine. In an anesthetized small canine preparation, atropine, injected intravenously, counteracted electrical stimulation of the right vagus. By varying stimulation strengths, the investigators[209] determined the minimum amount of atropine required to nullify a weak (0.1-0.4 mg atropine) or a strong (0.5-1.0 mg atropine) vagal stimulation. Thus, this study established for the first time a relationship between vagal stimulation and the amount of atropine required to cancel a prescribed influence upon the heart rate. Until this point, the discussion has focused on the effects of atropine on cardiac function in various experimental and human studies comparable to or in a state of rest. In 1930, Moore and Cannon[254] illustrated how external stress influenced heart rate prior to and following 1 mg per kg body weight of atropine in the unanesthetized cat. Following comforting rest (60-128 beats per minute), gentle, but firmly applied restraint of the cats caused a 241% mean increase in heart rate. After atropine, the baseline heart rate ranged from 176 to 240 beats per minute. The same restraint procedure elicited a 135% mean increase in heart rate. Even though the percent of baseline increase after atropine falls considerably below that observed without atropine, the heart rate during restraint following atropine (mean of 272 beats per minute) exceeds the heart rate in restraint without atropine (mean of 230 beats per minute). These observations will hold for future studies which will utilize other forms of stress. Atropine administered to dogs with fixed heart rates, between 136 and 210 beats per minute, did not affect ventricular contraction or refractory periods.[81]

Rather than initially pursuing the responses to atropine by category or targeted variables, I chose to concentrate on the initial era of concerted study (1916-1930) in toto. Not only did numerous studies unfold during this period, but possibly more importantly from the scientifically historical perspective, the aforementioned studies have been substantiated in numerous publications through to the present time. Although further additional knowledge about atropine did unfold subsequently, a majority of our knowledge concerning this drug's influence upon the cardiovascular system stems from this era.

III. ATROPINE, HEAR RATE, MYOCARDIAL BLOOD FLOW AND OXYGEN UTILIZATION

One of the major cardiovascular occurrences directly related to atropine involves the great increase in heart rate. Early experiments, described above, accurately defined this phenomenon, which many investigators have subsequently verified. In extensive exercise testing of horses, Detweiler[72] found that the horses fatigued readily following atropine. Higher doses of atropine, in particular, emphasized the exhaustion and weakness in the hind legs of horses. Detweiler could not relate the degree of fatigability

to changes in heart rate, blood pressure, respiration or temperature regulation in doses up to .24 mg/kg administered subcutaneously. Feinberg et al.[98] have defined a relationship for the product of heart rate and blood pressure to myocardial oxygen consumption. This relationship will hold where 0_2 extraction levels remain stable, which is not always the case, such as following epinephrine. Left coronary blood flow represents a sensitive indicator of left myocardial oxygen consumption. Since coronary stroke volume increases only slightly (<20%) and heart rate augments 242% during a given stress, while coronary oxygen extraction increases by <15%,[181] a fair postulate could equate a substantial degree of myocardial oxygen uptake to heart rate. Review of many studies has revealed a marked increase in heart rate following atropine administration in excess of 0.5 mg. In some studies aortic blood pressure rose moderately; in others, little or no increase took place. Hence, in looking at the major consistent cardiovascular response to atropine for a given heart rate at a given state of recumbent rest, standing rest or exercise, possibly, more myocardial oxygen utilization might occur for these conditions. With the plethora of available data showing heart rate augmentation following atropine, few studies have attempted to define the cost for the heart rate augmentation. Could the undefined fatigability observed by Detweiler result from reduced myocardial efficiency?

To adequately define this question an understanding of left myocardial blood flow and oxygen utilization in the presence of atropine should exist. As explained earlier, myocardial blood flow acts as the reserve for oxygen delivery to the cardiac muscle. Anrep and Segall, in 1925,[11] initially demonstrated an increased myocardial blood flow due to atropine. Their experiments took place in a modified *in situ* Starling heart-lung preparation under anesthesia. These first experiments demonstrated an atropine mediated coronary flow increase during vagal stimulation at the 7 mg dose level, but not at one-tenth that dose. However, the state of the dog and the level at which the vagi were stimulated made interpretation difficult. Rein, in Germany,[305] showed a doubling of left anterior descendens flow following intravenous infusion of 0.5 mg of atropine. Mean blood pressure rose from 90 to 105 mmHg, however, no mention of heart rate appeared. To further the confusion of this period, Naranya[263] in 1933, stated without evidence that atropine at "physiological doses" did not change coronary blood flow.

In the early 1940's, Essex and his colleagues published a series of papers relating atropine dosage to coronary artery blood flow in narcotized and unanesthetized dogs.[95, 96, 384] They used the thermostromuhr to register coronary blood flow. The thermostromuhr exhibited questionable sensitivity and linearity characteristics;[126, 337] however, in spite of such caveats they did present good evidence for an elevation of coronary blood flow following atropine. The duration of coronary artery blood flow response to atropine in contradistinction to the other drugs tested appeared much longer in duration (up to 49 minutes). The authors demonstrated that atropine causes a rapid increase in coronary blood flow. However, unlike the augmentation induced by other flow inducing drugs, following atropine the flow remains elevated at the initial level and only very gradually does this flow return towards initial levels. These authors never developed an extended time or half-time response of coronary blood flow to atropine. Such an extended period of elevated drug response, particularly when added to exercise stress, might very well explain the exaggerated debility observed by Detweiler.[72] In their initial paper[95] 2 mg of atropine fostered a 186% increase in left circumflex blood flow in one dog. Right coronary artery blood flow increased to a similar extent following atropine (4 dogs). An increased augmentation of blood flow occured with larger doses of atropine. However, two points restricted total credible acceptance of the aforementioned results, namely, the few tests run and the lack of concurrent heart rate data. In a second study[96] in unanesthetized dogs, the authors found that atropine caused an augmentation in heart rate and blood flow in the sympathectomized or unilaterally vagotomized (left) dog, but atropine showed no effect upon coronary blood flow, heart rate or blood pressure in the totally denervated heart. Blood pressure did not change appreciably in the sympathectomized dog following atropine. In a separate study,[384] 1.5 mg and 2.0 mg of atropine caused roughly the same percent increase in right and left coronary blood flow in the dog anesthetized with chloralose. However, the degree of augmentation did not reach the level observed in the unanesthetized dog. The reported elevations of coronary blood flow resulted mainly from increases in heart rate.

Gorlin and associates, in 1956,[121] undertook a study to ascertain the association of cardioacceleration

promoted by atropine to left myocardial blood flow and oxygen consumption in patients. They used the nitrous oxide method[86]—a method subsequently found to possess vulnerable sources of error[184]—for determining myocardial blood flow and the exremely reliable method of Van Slyke and Neill[365] to determine oxygen quantities. Gorlin's team found that 1-1.4 mg of atropine administered intravenously caused heart rate and blood flow to increase 140% whereas myocardial oxygen consumption rose to 132% of baseline. A good correlation existed between heart rate elevation and myocardial oxygen consumption. In these patients, left myocardial work did not change appreciably due to atropine, yet the oxygen consumption did; thus, efficiency in terms of oxygen utilization diminished from about 28% to 21%.

At the same time as Gorlin and associates initially described the excess myocardial oxygen utilization in humans due to atropine, Scott and Balourdas[335] described the same results in dogs anesthetized with either morphine and chloralose or diallylbarbituric acid (Dial) and urethane pentobarbital. Unfortunately, the data appeared as means and the control group consisted of considerably more subjects than did the atropine treated group; hence, precise interpretation of their results in certain respects proved difficult. In both treatment groups the dogs received 0.2 mg/kg of atropine, intravenously. Heart rate doubled, coronary blood flow augmented to a slightly lesser degree, and left myocardial oxygen utilization showed even less increase, though still appearing substantial. The slightly greater increases in coronary blood flow response with chloralose related to the greater work of the left heart accomplished with this anesthesia as opposed to those dogs studied under Dial and urethane anesthesia. Work increased after atropine in the dogs with chloralose (137%), but diminished to 86% of baseline in the dogs with Dial; likewise, mean aortic blood pressure increased to 114% under chloralose and decreased (92%) under Dial and urethane. Still in both groups left ventricular efficiency decreased following administration of atropine to 77% of baseline for the chloralose dogs and to 53% for the Dial dogs. Scott and Balourdas[336] confirmed their original observations using chloralose anesthesia in dogs, emphasizing the increased heart rate and myocardial oxygen consumption in the same state following intravenous atropine of an unspecified amount. In these latter experiments the mean heart rate augmentation (349%) exceeded that obtained in the initial study (249%). Yet, the level and percent increases of myocardial oxygen consumption appeared greater in the first study. Prior to stating unequivocally that the oxygen utilization augments after atropine, though not in a linear or even a totally positive relationship, one would have to examine data from individual subjects and know that equal dosages of atropine existed for all animals. Also, the reader must realize that the nitrous oxide method used to determine coronary blood flow in the previous three studies requires an extended steady state period and thus remains subject to error on this account and also due to the many technical hazards related to any gaseous technique. The studies of Gorlin and Scott's groups represent the only attempts, to date, to define the metabolic cost to the heart due to atropine. Unfortunately, neither study represents a valid sample of either the human or the canine populations and we still do not know what penalties atropine extracts from the heart during stress, in general, and exercise in particular.

Vatner et al., in 1970,[375] administered atropine (0.2-0.5 mg/kg) to the conscious dog. Subsequently, the left circumflex artery blood flow accurately monitored by an implanted Doppler flowmeter increased an average of 219% with an attendant 190% increase in heart rate and a 116% increase in central aortic blood pressure. The initial coronary vascular resistance averaged 2.23 peripheral vascular resistance units (PRU)[125]; following atropine, the coronary resistance dropped to 1.18 PRU. These investigators then stimulated the carotid sinus nerve and observed a further drop in coronary resistance to .96 PRU. The lack of information on individual responses or at least an indication of response variation detracts from the full potential for such a study. Perhaps the elevation in mean arterial pressure seen in this and other coronary studies could be attributed to heart rate elevation without a fully concomitant reduction in peripheral vascular tone.[267]

In 1974, Knoebel and associates[185] observed hemodynamic responses to atropine in normal male humans, ages 32-56. Even though attenuated heart rate responses appeared in the older individuals, a good correlation between heart rate elevation and myocardial blood flow increase took place ($r = .87$ by the least squares method) following intravenous administration of 1 mg of atropine. The subjects

rested in the supine without sedation during the study. Heart rate increased 118-192% above baseline (mean 152%) while myocardial blood flow concomitantly rose 110-196% (mean 147%) above baseline in response to atropine. During these studies, arterial pressure remained essentially at baseline (102 ± 5% SD) whereas cardiac output rose slightly (114 ± 15% SD). Stroke volume, however, decreased to 76 ± 13% SD of baseline following atropine. All individuals showed this decrease. The reduction in stroke volume in concert with an elevated heart rate following atropine has occured consistently in many studies, one of which I cited earlier and in more which will be referred to later. This reduction of stroke volume does not occur in stress situations such as exercise, except at the beginning of a race or during a startling encounter and then only during the initial few beats following the stimulus.[78, 174, 181, 303, 322, 323] Hence, this consistent observation must substantiate a unique response to atropine. The reduced stroke volume represents a perplexing occurrence with respect to the study in question,[185] for contractility increased 120 ± 15% SD (in all but one individual whose contractility registered 97% of control), whereas total peripheral resistance diminished (90 ± 11% SD) (in all but one individual whose resistance rose to 108% of baseline). Possibly, peripheral systemic pooling[174] precluded reduced return following atropine administration, for pulmonary capillary pressure remained essentially the same[64] and right atrial pressure dropped from 7 mmHg to 4 mmHg.[243] To further substantiate a relationship between the reduced stroke volume and peripheral vascular blood pooling, Weissler and associates[385] observed an increase in the depressed stroke volume that appears following atropine, upon increasing splanchnic and leg resistance by inflation of an antigravity suit about these areas in man, while he stands. Further, with the increased stroke volume, the reduced central blood volume increased, also. Left heart minute work increased in seven of ten individuals while remaining slightly under baseline in the other 3 persons. As a group, left ventricular work increased 117 ± 19% SD. In the resting state, elevated myocardial blood flow inferred a reduction in efficiency following atropine. The plethora of myocardial blood flow attributed to atropine, yet not totally accounted for by the work of the heart, may resemble the over repayment of debt phenomenon seen during reactive hyperemia in the coronary bed.[281] The precise mechanism of this response evades definition.

In a comparison of supine rest to controlled supine ergometry in 6 young human males, Kahler and associates[165] defined hemodynamic and oxygen utilization changes prior to and after atropine. I estimated left myocardial oxygen utilization[98] in order to associate the other variabiles with an estimate of this parameter. Following administration of atropine at rest, heart rate definitely rose in all subjects. Stroke volume dropped to 79 ± 16% SD of control in all but one subject, whose baseline stroke volume appeared lower than that of the others. Consequently, cardiac output remained the same or dropped in 2 of 6 subjects (with the lowest stroke volume) whereas cardiac output rose in the remainder of the treated subjects. The augmented cardiac output observed by Kahler et al. runs counter to previous reports. Although stroke volume diminished, the amount of reduction, in several cases, did not compensate sufficiently for the augmented heart rate. Total body oxygen consumption did not change. However, the computed values for myocardial oxygen utilization showed an unequivocal increase in myocardial oxygen utilization following atropine ($p < .005$). Minute work of the heart increased even though stroke work decreased. Such observations clearly indicate that atropine causes a marked decrease in cardiac efficiency at rest (73% baseline rest) ($p < .005$). In assessing the effects of atropine upon exercise, a clear-cut comparison does become more difficult. In order to ascertain a fairly representative perspective, one must look at both the change of exercise values with respect to the baseline values and also the comparative aspects of exercise response with and without atropine. In these particular experiments atropine caused heart rate to augment to a slightly higher level than did exercise without atropine. Therefore, as one might expect from the previous statements, heart rate elevation does occur with exercise, following administration of atropine, but the ratio of increase appeared considerably less (1.7 vs. 1.3). Stroke volume decreased less during exercise, after atropine, than at rest with atropine. Due to the overall elevation of heart rate with a reduced stroke volume observed in exercise after atropine as compared to the same response prior to atropine, cardiac output for both exercise tests attained the same level with no statistical difference. Myocardial oxygen utilization values, however, appeared greatest during exercise following atropine, even though minute work showed no significant difference between

the two exercise tests and stroke work diminished in a manner similar to that observed before atropine. During exercise, the efficiency of the left myocardium decreased by 18% after atropine ($p < .025$) even though the work of the heart did not appreciably change after atropine at the same level of exercise. Myocardial oxygen utilization (as calculated),[98] if true, increased after atropine. Since heart rate represents one of the products used to estimate myocardial oxygen utilization, we cannot properly ascribe the increased oxygen utilization to heart rate increase, even if this might represent a correct assumption.[181]

Studies by other groups, in various species, have shown that atropine causes heart rate elevation[25, 54, 174, 243, 312] and little change in cardiac output, though the latter usually appears slightly elevated.[25, 174] Cardiac output remains relatively unchanged due to the pronounced drop in stroke volume.[25, 174] In addition to confirming results obtained by others, Daly and coworkers[64] showed that right atrial pressure and pulmonary artery pressure decreased after atropine while pulmonary capillary volume remained unaffected. Evidently, pulmonary arteriolar tension must decrease after moderate doses of atropine. Apparently, oxygen intake remains fairly constant for a given level of exercise, whether the mammal has received atropine or not; however, under the influence of atropine an exhausting exercise does reduce significantly ($p < .005$) oxygen intake when compared to the same effort without atropine.[312] Three of 5 subjects became exhausted sooner when tested approximately 1 hour and 40 minutes after administration of 2 mg of atropine intramuscularly. Robinson and coworkers[312] demonstrated that atropine only began to influence oxygen intake when no further vagal influence upon heart rate appeared; in other words, control heart rate levels and heart rate levels after atropine attained identical levels during an exercise performance sufficient to promote exhaustion within 5 minutes. The mechanism for the observed drop to 90 ± 6 SD percent of control oxygen intake remains unknown.

IV. ATROPINE AND THE AUTONOMIC CONTROLS OF CARDIOVASCULAR FUNCTION

In anesthetized and unanesthetized dogs, small doses of atropine methylbromide, a compound which does not cross the blood-brain barrier, slowed the heart.[194] In another study, using the Langendorf isolated heart preparation,[307] small doses of atropine sulfate promoted a reduction in heart rate. These studies demonstrated a direct effect of atropine upon a target tissue. Possibly when a physiological event eliminates neurally mediated muscarinic effects, such as in the exhaustive exercise referred to above, antagonists such as atropine sulfate, which do not ordinarily show agonist properties, do exert some degree of muscarinic effect. Should such a pharmacological event take place, atropine could possibly cause bronchiolar constriction, thereby limiting oxygen intake. Daggett and associates[61] demonstrated that atropine blocked the reduced contractility and elevated left ventricular and diastolic pressure due to parasympathetic stimulation in the anesthetized dog. They found that atropine, per se, does not cause an augmentation of left ventricular contractility. Upon simultaneous blockade of the competing autonomic nervous systems, Nordenfelt[278] observed that heart rate, cardiac output, mean ejection rate and blood pressure did not reach the levels noted for a given level of exercise with a functional autonomic nervous system. Therefore, the observed augmented values for these variables following parasympathetic blockade probably represent an additive effect of the sympathetic system as well as the reduction of inhibition by the parasympathetic nervous system.

We have followed the cardiovascular responses to atropine at rest and during exercise. Does atropine have an effect upon the reflex adjustments from the supine to the verticle position? A study by Weissler and coworkers[385] indicated that atropine does influence baroreceptor-mediated responses. Following atropine, heart rate accelerates to a significantly greater degree ($p < .05$) in subjects with their heads tilted 60° than in subjects in a recumbent position than those subjects tested without atropine. One divergent finding by this group concerned the marked increase in recumbent cardiac output following 2 mg of intravenous atropine. Only one subject in six responded, as previously reported, with a marked heart rate increase, reduction in stroke volume and slight (< 130%) cardiac output increase. Deletion of this one subject from evaluation caused the remainder of this treated group to show an average increase in heart rate of 169% above control, and cardiac output 186% above control with stroke volume 110% above control. Discounting the latter 2 discordant results, the authors[385] found that those individuals in

the 60° tilt group showed no significant difference ($p < .05$) in the percent of heart rate elevation after atropine than that attained in the recumbent group. However, a marked difference appeared in cardiac output, which increased 107% above control following atropine in the tilt group. The greatly reduced cardiac output occured because the stroke volume only attained 61% of control following atropine in the tilt group. Response to atropine in the tilt group more closely resembled previous studies inasmuch as the increased heart rate, coupled with the limited cardiac output increased to under 130% of control. Of course, this still does not determine the responses to atropine exhibited by the 2 positions.

I have not discussed, directly, the effects of atropine upon the baroreceptor and chemoreceptor buffer systems. These buffer systems mediate their actions through centers in the medulla oblongata. The afferent part of this system, the parasympathetic fibers of the glossopharyngeal nerve, correspond to stimulation by slowing of the heart[69, 70, 117, 202] and reduction in left ventricular inotropic forces[69, 70] usually observed following elevation of pressure in the region of the carotid sinus. Due to the increased parasympathetic responses associated with stimulation of the carotid sinus, cardiac output and stroke work fell with an elevation of mean atrial pressure attributed to a depressed atrial contractility.[325] Hackett and associates[129] found that atropine blocked the coronary vasodilation response brought about by chemoreceptor stimulation. They showed that carotid sinus stimulation utilized the same parasympathetic vasodilator pathway described for the chemoreceptor response, but also appeared to function by moderation of the peripheral coronary vasoconstriction tone. They[129] corroborated the previous observations of cholinergic vasodilation of the coronary blood flow by Feigl.[103] The observed coronary vasodilation due to active parasympathetic stimulation and change in vasomotor constriction tone took place in anesthetized dogs.[103, 129] Vatner and associates[375] showed in the unanesthetized canine that carotid sinus nerve stimulation caused a reflex vasodilatation of the coronary bed that occurred to a further extent in the presence of at least 4 mg of atropine. With atropine alone, the computed resistance of the left circumflex artery (1.18 PRU) amounted to 53% of baseline, whereas during carotid sinus stimulation in the presence of atropine, the resistance dropped further to 0.96 PRU. This group concluded, by additional alpha adrenergic blocking tests, that the drop in resistance occurred due to a reduction in sympathetic constrictor tone. Although agreement of a coronary dilator response to carotid sinus stimulation prevails, the afferent mechanism will require further clarification. Possibly all aspects of coronary vascular control and reflex heart rate adjustment during orthostatic reflex testing should come under scrutiny since the studies of Neto et al.[272] have demonstrated a delayed beta sympathetic heart rate increase after a 70° head tilt (1-5 min). These authors corroborated findings in Chagas disease patients (no parasympathetic S-A node control) with studies in normal patients after atropine. Both groups showed an attenuation of heart rate response, after removal of parasympathetic controls, when administered a beta adrenergic block. Weissler's group[385] certainly showed a response change due to position and atropine. Hackett's group[129] showed some reservation and remained somewhat equivocal with regards to the afferent mechanism and thus the importance of atropine upon the baroreceptors' mediated response. Levy's group[69, 70, 202] showed that the carotid sinus reflex reduced inotropic strength of the left myocardium. The observations of Knobel et al.[185] showed that the index for increased inotropy rose significantly ($p < .005$) following atropine. Surely, the relationship of inotropy to atropine probably relates to heart rate (these two variables changed in the same direction) and not to any muscarinic or antimuscarinic effects upon the ventricular myocardium. A direct effect of atropine on the ventricular myocardium appears doubtful due to the lack of acetylcholinesterase containing nerve fibers in the myocardium; these fibers surround perivascular structures and densely populate the conduction bundles of the ventricles.[175, 179] Stimulation of parasympathetic nerves to the heart caused at most a 5%-7.5%[111, 135, 213] reduction in ventricular contractility at a constant heart rate. The reduction in ventricular contractility did not evince consistent reproducibility.[135] What small degree of reduced contractility that did appear took considerably longer to manifest itself than after a comparable stimulation and reduction of atrial contractility. However, claims exist that link parasympathetic stimulation to negative inotropy.[142] Many of the models used to demonstrate negative inotropy do not represent sound physiological preparations, hence further studies to elucidate the parasympathetic ventricular function will be required prior to gaining more definite knowledge about the relationship of atropine to ventricular contractility. Miller and associates[243] showed that atropine

(2 mg) caused a peripheral vascular dilation with a drop in systolic blood pressure and an increase in heart rate. They demonstrated further that the resultant drop in pressure and compensatory heart rate increase ameliorated when they elevated abdominal and leg resistances by activation of aviation antigravity suits on human subjects.

V. ATROPINE AS AN AGONIST

Intramuscular injection of atropine administered manually or by an autoinjector[232] infuses atropine deep into a large muscle mass. Unless the needle tip lodges into a vessel, dispersion and distribution of atropine shall allow for low initial levels.[255] As described in an earlier paragraph, low levels can promote a biphasic response. Hence, initially individuals may experience a slowing of the heart instead of the familiar rapid increase of heart rate usually associated with atropine. Since the 1920's numerous groups[41, 66, 67, 163, 194, 217, 238, 261, 307, 333] have confirmed the earliest observations that small doses (usually limited to less than 0.5 mg of atropine in human or dog) promote a reduced heart rate within a short period of time lasting for a brief duration (usually 1 minute or less). These studies continue to appear in the literature through the present time.[333] Mention of the mechanism either does not appear or remains for the most part undefined. Heinekamp[141] proposed that small doses of atropine caused stimulation of the vagus at the level of the dorsal motor nucleus. Further studies to prove this hypothesis have not appeared in the literature. However, in support of such a hypothesis, Feldberg[99] and Miller and associates[242] found that synthesis of acetylcholine takes place in the brain with the greatest production occuring in the cortex[99, 242] and with the next greatest production taking place in the brain stem.[99] However, in comparison with peripheral nerve endings, the concentration of acetylcholine found in the brain appeared low.[100] To further lend credence to Heinekamp's hypothesis, Molenaar and Polak[252] found that atropine stimulated production of acetylcholine in slices of rat cerebral cortex, particularly in the presence of $CaCl_2$. Two questions become evident: First, does atropine cause production of acetylcholine in the dorsal nucleus or even in the brain stem? Secondly, does acetylcholine act as the initiator of afferent impulses in the medulla in general and the dorsal nucleus in particular? Feldberg and Vogt[101] found that one of the highest levels of acetylcholine synthesis occurs in the dorsal nucleus. More recently, investigators have shown that small quantities of atropine, $3 \times 10^{-7} \mu g$/gram of rat cortical tissue, can stimulate a 162% increase in acetylcholine production.[238] Evidently, the amount of atropine required and the synthesis and release of acetylcholine do not show a close correlation.[286] At this time one can only surmise that a similar atropine-acetylcholine relationship exists in the dorsal nucleus thereby initiating an afferent parasympathetic inhibition of heart rate due to small doses of atropine. Not only can the blood level of atropine serve as an index of heart rate decline, but a slow rate of infusion also can cause a reduction of heart rate even though blood levels increase.[42] The response to atropine might also depend upon the rate of arrival at the muscarinic receptor site.[358] In contradistinction to a central nervous system response to low concentrations of atropine inducing a parasympathomimetic response upon heart rate, more recent studies have presented evidence substantiating a direct muscarinic effect for small doses of atropine directly upon the S-A node.[194, 307, 358] A dual pharmacological role may exist with regard to small levels of atropine upon heart rate, consisting of a strong stimulation of the dorsal motor nucleus while simultaneously blocking the peripheral vagal inhibition at the pacemaker site.[343] This group attributes a 50% peripheral blockade by .06 mg of atropine whereas 1 mg of atropine causes greater than a 90% blockade of peripheral vagal inihibition at the S-A node.

VI. EXERCISE, AGE AND ATROPINE

In any study involving exercise and atropine, particular attention to age becomes important. A consensus prevails that heart rate response following administration of atropine attains the highest levels in the early decades of human life, with the peak response probably falling somewhere between 15 and 40 years of age[39, 66, 171, 237, 261] and most likely appearing in the third decade. A second period of a more augmented heart rate response though at a lower level occurs in the sixth through the eighth decades

of human life.[39, 68, 237, 261] Less information appears with regards to the impact of age upon blood pressure following administration of atropine. Possibly, such findings remain limited due to the observations of elevations, depressions and no changes in blood pressure following atropine in different studies[25, 54, 64, 72, 136, 185, 243, 261, 335, 336, 341, 351, 375] where the authors did not attempt to associate atropine induced pressure changes with age. Carrow and coworkers[39] observed a variable, age dependent systolic blood pressure response in anesthetized patients following intravenous administration of atropine. They found that the greatest elevation in systolic pressure occurred in the 2nd and 8th decades, yet the augmentation difference between the highest group (2nd decade) and the lowest group (3rd decade) was 7½%. To further add to this muddled picture of pressure responses, some groups looked at mean pressure responses, others observed either systolic or diastolic pressure, and one evaluated pulse pressure. Work at the Harvard Fatigue Laboratory[73] has shown that an extremely high negative correlation exists ($r = .96$) between the age of an individual and the heart rate response to a standard, mild exercise test. In a 28 year study on one individual, the same heart rate-age relationship existed;[73, 74] as the heart rate dropped with age for a given exercise level, the maximum oxygen utilization diminished, also. The effects upon responses to atropine and exercise definitely warrant consideration of age, when one proposes to study the effects of atropine upon exercise performance.

VII. A CASE FOR TACHYPHYLAXIS

In the unanesthetized dog, Donald et al.[79] found that 2 mg/kg of atropine augmented heart rate by 282%. Subsequent testing of the same dogs elicited a diminished response on days following the initial challenge. By the fifth day, the same dose promoted a 226% heart rate increase above baseline. The initially high heart rate response (282% vs. 226%) probably represented a differential attributed to sympathetic response. However, no specific results appeared which established that the initial challenge represented an augmented sympathetic response that did not subsequently occur. A parallel experiment by Donald and associates[79] showed that following cervical vagotomy in the conscious dog heart rate attained a high level similar to that seen after atropine, except the level remained sustained for 30 minutes instead of decaying; upon administration of propranolol the heart rate fell by an average of 19% in 3 dogs. This observation would indicate that the initial overshoot in heart rate following atropine on the first day could originate from the vagolytic action of atropine plus sympathetic stimulation. In an interesting study by Duchene-Marullaz and associates,[82] eleven free-ranging dogs were administered atropine by mouth (10 mg/kg) initially followed by 200 μg/kg intravenous injections of atropine at 8 and 23 hours; they showed a reduced heart rate response to the subsequent doses. Not only did each response diminish in magnitude, but also each ensuing recovery towards baseline took place at a more rapid rate. The authors do not relate these observations to a tachyphylaxis nor to an increase in enzymatic response. In fact, they ascribe their observation to an unknown mechanism. In the strictest sense of the word by definition tachyphylaxis represents: "diminished response to later increments in a sequence of application of a physiologically active substance (as the diminished pressor response that follows repeated injection of renin)."[113] Evidence above supporting tachyphylaxis in the experiments of Duchene-Marullaz[82] appears more probable due to the overt tachyphylaxis observed in the stomach[295] and intestine[124] of the dog after repeated doses of atropine. To preclude tachyphylaxis type responses, Gangway et al.[112] allowed four days between the testing of atropine on conscious dogs without offering a reason for their approach. Even though these few studies surely do not offer unequivocal evidence of tachyphylaxis after repeated atropine administration, nevertheless they do present the possibility that atropine might generate such a response. Since acetylcholine and atropine exhibit a competitive antagonism for the receptor site,[119] possibly a negative feedback system exists which could promote the synthesis and/or release of additional acetylcholine following additional serial doses of atropine. Kelbinger[189] has shown that such a mechanism exists in the guinea pig smooth muscle myenteric system, but not in the chicken, guinea pig heart, or guinea pig bladder. However, much more work must appear in more species and organs utilizing more concentration combinations before we can relate such a mechanism to tachyphylaxis. Further work likewise must take place to illustrate whether

or not repeated doses of atropine do cause tachyphylaxis. In order to establish a protocol using conscious animals, investigators must either guess, as previously done,[112] or determine a temporal bases for the administration of multidoses of atropine without causing a precedence of dependent influence.

VIII. ATROPINE KINETICS

What is the duration of response following atropine? When asking this question usually one also desires to know the time to peak response. These issues represent desired information for all medicaments, not just for atropine. Heart rate change represents the major response used to answer these questions for atropine. Besides duration, per se, atropine response can be interpreted by the total dose and route of administration.[255] In all individuals tested with intravenous atropine, Conrad found a good correlation between the dose administered and the ultimate increase in heart rate.[46] A good correlation exists between the extent of heart rate response and the level of atropine in the blood level of the dog when one administers at least 0.2μg/kg of atropine intravenously.[42] However, when the same dosage extended over a period of one hour in the dog, the initial tachycardia would decrease in spite of a buildup in the atropine blood level.[42] Immediately, the reader can understand that the rate of administration can influence the direction and magnitude of heart rate change due to atropine. In assessing the data, with respect to man, Robinson and associates[311] found that dogs attained a maximal level of dose response in about one-half the time that man does. Possibly this point could be attributed to the more rapid circulation time of the dog,[390] which approximates one-half that of man.[308] Therefore, a direct relationship may exist between the amount of time to reach maximal response to atropine and the circulation time required between the same foci in more than one species, as determined by the same indicator technique. In humans not only will a larger dose of intramuscularly injected atropine attain a higher initial heart rate response, but the duration of the nearly maximal heart rate level also appears related to the dosage administered.[180] Thus, not only does overall response last longer, but the level of peak response also remains longer, in a dose related manner.[180] The results following subcutaneous administration of atropine in the horse did not manifest a dose relationship to peak heart rate, duration and return to baseline heart rate levels.[72]

Oral doses of atropine promoted heart rate responses of the longest duration,[82] exceeding 8 hours duration in dogs. Intramuscular doses in man peaked within 15 minutes[50] and lasted 3 to 5 hours[50, 180, 236] with a slight drop below control noted in one study[236] 7 to 8 hours after injection. Following subcutaneous injections in man, the maximum heart rate response occurred within 20 to 30 minutes,[274] the same time frame observed for intramuscular injection of atropine. Detweiler[72] found the duration of atropine effect in horses following the subcutaneous route, lasted about 5 hours. The initial bradycardia following the same dosage in man occurred 30 to 40 times sooner after intravenous administration than after a subcutaneous injection.[135] Intravenous injections of atropine peaked within 5 minutes in the dog[79] and within 60-90 seconds in man,[41, 51] with a lesser dose. Responses to intravenous atropine lasted from 2[51] to 5[120] hours in man. Knowledge of peak heart rate response and duration remains incomplete, particularly since a well-controlled comparative study defining dosage effects via the four major pathways for introduction in one specie does not appear in the literature. Summarized briefly, oral administration causes the longest duration of response, whereas intramuscular and subcutaneous injections produce responses which generally peak later than those produced by intravenous administration, but have durations of response possibly only slightly longer than those produced by doses administered intravenously.

IX. ATROPINE, THE ENVIRONMENT AND EXERCISE

Atropine, in sufficient amounts to produce a vagolytic effect, augments the heart rate at any given level of activity below maximum effort. At cool temperatures, 70°F or less, at rest or during mild exercise (3 mph), 2 mg of atropine does not markedly affect the ability of man[49] or dog[311] to function in a manner similar to that without atropine. Looking at effort and environmental indices of temperature and relative

humidity alone does not present an accurate picture of the effects of atropine upon performance. Such a statement rests mainly on the two comparative studies where men received the same amount of atropine and exercised at nearly the same rate at comparable temperatures and humidity. In one study, men walked on a treadmill at 3 mph at 86°F and 80% relative humidity for two 45 minute periods broken by a 15 minute rest.[115] Two of the men completed the test in a manner similar to control or exercise with atropine at 70°F and 60% relative humidity. Unfortunately, the authors do not describe the progress of the one individual who developed difficulties; the authors imply that the individual did complete the intended exercise regimen but not in the designed time period.[49] In another study the same group of men should have marched for 115 minutes at 3.3 miles per hour, on successive days with the ambient temperature at 83°F and the relative humidity at 56% and 59%.[310] On the first day, without atropine, all 33 men completed the test with an average peak heart rate of 111 beats per minute, an average rectal temperature of 100.6°F, and an average sweat loss of 363 grams per M^2 body surface area per hour. In contrast, following 2 mg of atropine intramuscularly, some prior to and others 30 minutes into the march, only 5 of 18 men receiving atropine during the exercise finished the prescribed time course, whereas 10 of 15 receiving atropine prior to exercise finished the specified 115 minute march. The men quit from 45 minutes to 100 minutes after beginning the march. Upon receiving atropine, prior to exercise, the men attained average heart rates of 177 beats per minute, had an average rectal temperature of 102.8°F and lost 210 grams per square meter of body surface area of sweat per hour by the conclusion of their march. Those receiving atropine after marching for 30 minutes had an average end-of-exercise heart rate of 159 beats per minute, an average rectal temperature of 102.1°F and shed an average of 231 grams of sweat. On an average, men receiving atropine after 30 minutes of exercise, then continuing their march, produced 12% less sweat than those receiving atropine prior to exercise.[310] The end results tabulated for both groups above do not accurately describe the increased rigors experienced by the group who received atropine during the march as compared to those who received the same amount of atropine prior to the march. The comparative number of non-completions does attest to added rigors of the group receiving atropine during the exercise test. For an unexplained reason, pre-exercise appears markedly to exacerbate the stress of exercise following atropine. A further comparative study of those completing the march versus those who failed for both groups furnishes more conclusive evidence for the added heat stresses endured by those receiving atropine after exercise had begun.

Those individuals not completing the march, who had received atropine prior to the 115 minute march[310] had a significantly higher heart rate ($p < .005$) at the end of exercise (121%) than those who did march for 115 minutes. Sweat production for those not completing the march amounted to 64% of that produced by those who completed the march ($p < .005$). The percentage of sweat production for the exercise period with atropine, compared to the exercise period without atropine, amounted to 45% in those who failed to complete versus 67% in those individuals completing the 115 minute walk ($p < .01$). Rectal temperatures in the group that finished the exercise averaged 101.6°F, whereas the average for the individuals failing to finish came to 103.0°F ($p < .005$). Upon evaluating the exercise results from those persons administered 2 mg of atropine intramuscularly, 30 minutes into the exercise, a greater percentage of these people failed to complete the march. An average heart rate of 186 beats per minute represented the end exercise rate in people who failed to finish, whereas those who did finish had an average heart rate of 153 beats per minute (122%, $p < .005$). Sweat production appeared somewhat greater, on an average, in those failing to complete the test in the group who received atropine during the exercise, than in the first group who failed to finish the test. In the group who took atropine after the exercise began, those who finished had a significantly lower average rectal temperature (102.0°F) ($p < .01$). A much higher percentage of individuals (67%) finished the exercise who received atropine prior to exercise, than those who received atropine during the exercise (22%). Of those who finished the exercise in both groups total production of sweat in the group taking atropine prior to exercise comes to 260 grams per meter square of body surface area per hour ($g/M^2/hr$), while those receiving atropine during the exercise produced an average of 226 grams of sweat per M^2/hr. Those finishing the exercise test had a slightly, yet significantly lower rectal temperature (101.6°F vs. 102°F)

than those individuals who could not finish the exercise. When looking at the failures from both groups, it becomes difficult to ascertain why the group who received atropine during the exercise test did not fare as well as the other group. Heart rate and rectal temperatures (with some exceptions) remained lower whereas a greater sweat production (with some exceptions) usually occurred in those individuals who completed the 115 minute march than in those individuals who quit marching. Evidently the reason for failure to complete the exercise regimen does not necessarily relate directly to compensatory changes associated to heat loss. The material in this paragraph represents a reanalysis of the data in a form which differs from the author.[310] One possible explanation for the divergent results involves the more extensive peripheral pooling of blood in the legs and splanchnic areas of persons receiving atropine during the run.[25, 166, 243, 385] Man cannot compensate for splanchnic pooling[14, 85, 379] by splenic infusion of blood during exercise, as is the case in the dog.[16, 17, 18, 376] Hence, extensive pooling in the splanchnic region[166, 243, 385] dependent peripheral vascular structures[166, 243, 385] and the skin[117] might have come about by the injection of atropine during the exercise, thereby precipitating an inefficient vascular system in a majority of the tested people. This suggestion coupled with an inefficient cardiac function, related to earlier, might have caused the individuals exercising to cease marching, for they could no longer cope with the combination of heat and exercise stress. Kalser and coworkers[166] offered several possible mechanisms whereby atropine might cause significant vascular blood pooling. Among those causes entertained, they included ganglionic antagonism of adrenergic-induced peripheral vascular tone and/or direct relaxation of peripheral vasculature. Apparently, further future studies, of appropriate design, will define the basis of atropine dependent pooling in man.

In a comparative study of heat loss in man and dog[311] at 40°C and 33% humidity, one dog received the same dose of atropine per unit body weight as administered to 6 men and exercised at the same rate (3.5 mph) for 2 hours on a treadmill. The heart rates for both species appeared very similar; the magnitude of rectal temperature increase appeared greater in man, however, the rectal temperature in the dog registered higher, yet the resting temperature of the dog usually registers 1.5°C higher than man. The 1 dog lost more fluid per square meter of body surface than did the men.[311] Certainly, many more studies must appear before one could draw a good comparison between man and dog under various environmental conditions with equivalent doses of atropine.[56]

In man, .04 mg/kg of atropine causes almost a complete block of perspiration. Although dogs do not have eccrine sweat glands about their body, except on the exposed paws, they do possess apocrine sweat glands[12] which do not participate in a centrally mediated sweat process due to exercise heat, but do respond to locally mediated stimulation due to local heat build-up. The latter mechanism does not play a major role in heat loss control during exercise. As commonly known, the dog effects most of its heat exchange by salivation and rapid air exchange, or panting, over a lolling, dilated tongue.

Very little work appears which defines the effect of acclimatization upon the responses to atropine in mammals in a warm or cold environment. Apparently, acclimatization ameliorates the influence of environmental extremes (35°F, 95°F) upon the lethality of atropine to mice, for the ratio of survivors to a mean LD_{50} dose of atropine rose dramatically in both hot and cold climates after 30 days acclimatization.[109] Probably a much shorter period of acclimatization would have sufficed. In the studies on man, heart rate, sweat, and other compensatory mechanisms affecting temperature response reacted extremely rapidly, within 4 days, and reached near maximum adjustment by 12-14 days.[320] The cardiovascular adjustments to heat acclimatization qualitatively appear the same as those noted after atropine; namely, reciprocal changes in heart rate and stroke volume with very little change in cardiac output and aortic blood pressure.[320] However, during acclimatization heart rate falls, whereas stroke volume increases. This manifests the opposite response from that observed after atropine. Possibly, when atropine administration occurs in the absence of acclimatization the increased heart rate and reduced stroke volume cannot compensate for a combined pooling of blood in the splanchnic region, lower extremities and the skin, thereby leading to a markedly reduced capacity for exercise function in a hot climate. Much more experimental work must take place prior to our obtaining a clear-cut understanding of the effects of atropine upon the acclimatized and unacclimatized human subject, particularly in a hot, humid climate.

X. PARASYMPATHETIC INNERVATION OF THE HEART

The review to this point has expanded the coverage of the traditionally known actions of atropine upon heart rate and some of the consequences to its elevation and at times depression. Most contemporary textbooks of pharmacology[75, 119] succinctly touch upon this atropine influence on heart rate. However, the mechanisms, anatomy and physiology associated with heart rate responses have received extensive study within the past forty years, with the most intensive effort coming since 1960. A major impetus for this pursuit, particularly since 1970, stems from a dire need to understand and control the bradycardia which can occur following a myocardial infarct using atropine. The bradycardia can lead to a fatal outcome. Some of the clinical experiences and clinically oriented studies using or not using atropine have added greatly to our present knowledge about cholinergic influences on the mammalian cardiac conduction system. Even though this review mainly concerns itself with the effects of atropine upon the heart, I feel strongly that a review of anatomical and physiological, as well as pharmacological backgrounds concerning impulse generation and subsequent conduction must appear. To substantiate this feeling, let it suffice at this point to state that much controversy and lack of concrete functional knowledge regarding the peripheral Purkinje system function and the associated autonomics remain for further elucidation. Until we know and understand this body of knowledge, we cannot possibly understand the full impact of muscarinic doses of atropine upon the heart. None of what appears in the following section will represent the overall embodiment of published information on any particular facet. The author takes full responsibility for such an approach and can only direct the interested reader to more comprehensive reviews and published symposia.

For an extensive anatomical description of the major preganglionic parasympathetic rami to the heart, the reader should turn to the work of Randall and Armour.[302] Considerable species differences occur in preganglionic parasympathetic distribution to the heart.[302] For a more definitive physiological understanding of parasympathetic pathways that influence cardiac function in the dog, stimulation studies described by Randall and Armour[300] will furnish this information. By differential chemical ablation of vagal branches, using phenol, Randall and coworkers[299] demonstrated by vagal stimulation discrete and independent parasympathetic efferent pathways to the S-A and the A-V nodes. Evidently, bilateral neural control prevails, that is, right vagal impulses influence the right heart whereas left vagal impulses influence the left heart. Such an anatomical alignment does not function in an exclusive manner.[300] Readers desiring further information about parasympathetic pathways to the heart may find such in the following references:[142, 173, 204, 207, 246, 247, 248.]

A. PARASYMPATHETIC INFLUENCE UPON MYOCARDIAL INOTROPY

Muscarinic effects upon the heart due to parasympathetic stimulation have been reported to have negative effects upon cardiac ventricular inotropy and chronotropy. The effect upon ventricular inotropy remains controversial[142] since the experimental preparations may not approximate conditions in the intact, viable, conscious mammal.[30] Not too long after DeGeest and associates published their paper[69] stating that vagal stimulation produced a negative inotropy, Braunwald et al.[30] called attention to the possible inappropriateness of the model as stated above and the same warning holds at the present time. Contrary to the study of DeGeest and coworkers,[69] several papers did show that the effects of vagal stimulation in the anesthetized canine preparation with a fixed heart rate,[61] in the blocked heart,[213] and the anesthetized canine preparation solely[212] did not effect inotropy. One earlier study did show a reduction in both auricular and ventricular (to a lesser extent) inotropy following vagal stimulation or acetylcholine administration[45] in unison with a reduced chronotropy. Since the DeGeest et al.[69] study appeared, a plethora of subsequent papers[26, 53, 61, 71, 135, 202, 203, 213, 271, 283, 290, 291, 292, 301, 345] has corroborated their findings. Due to the nature of this review, a detailed account of each protocol and its various, particular features would seem imprudent. Generally, all studies involved utilization of the open chest dog. Usually, the investigators paced the hearts between 150-200 beats per minute. In some experiments, such as the initiator of this series,[69] not only did the experimental protocol call for a paced heart

plus vagal stimulation, but also utilization of an isovolumic ventricle. The authors attributed the reduced inotropy, or contractility of the ventricles, to a direct vagal effect upon the ventricular muscle. Atropine either ameliorated or reversed the inotropic reduction following vagal stimulation.[61, 71, 20, 290, 291, 298, 301] During this period, Furnival and associates[111] did not elicit a negative inotropic response with vagal stimulation. Randall and coworkers[301] demonstrated a reduction of contractility in all four chambers; however, this occurred with a reduction in heart rate. In another study by this same group[53] vagal stimulation either with or without pacing caused a reduction in contractility; without pacing the heart rate fell, also. Randall and Armour[300] observed that regional reduction or suppressions of myocardial contractility generally occurred with an accompanying reduction in chronotropy.

In testing the effects of vagal stimulation upon the anesthetized duck's heart, which showed profound depression in peak systolic pressure with an increased end diastolic pressure, Folkow and Younce[107] demonstrated an interesting interplay between pacing and vagal stimulation. During the period of pacing of the heart without vagal stimulation, peak left ventricular pressure remained steady with periodic oscillations. When they stimulated the vagus while pacing the heart, the peak ventricular pressure slowly decreased to 86% of control within 18 seconds. Upon cessation of pacing with continuation of vagal stimulation, the heart rate slowed considerably and peak systolic pressure increased to 110% of control immediately. Upon reinstitution of pacing, peak systolic pressure fell precipitously to 69% of control. Finally, after release of the vagal stimulation, with continued pacing, peak ventricular peak rose to 123% of control within 10 seconds. This study demonstrated an inotropic reduction due to vagal stimulation; however the augmentation of inotropy with continued vagal stimulation without pacing surely suggested an interplay between the 2 electrical stimuli upon the heart. A hemodynamic heterometric or Starling-Frank mechanism could have promoted the augmented inotropy described in the previous sentence. Does such an interplay carry over to the mammalian experiments described above? Certainly, the explanation to this question remains unknown, but will require an answer prior to acceptance of the results of the mammalian studies as representative of normal autonomic function in the unanesthetized, intact, uncompromised heart of mammals. Atropine blocked the reduced inotropy observed in the duck. Although the mechanisms remain unknown for the studies outlined above, one might think of a negative Bowditch type response[62, 63] even though some studies included pacing. The negative Bowditch response could possibly result from an accumulated surplus of the negative inotropic effect of activation (NIEA).[186] The NIEA may be a manifestation of an outward extracellular movement of calcium ions.[172] An interaction of pacing and vagal stimulation might cause a reduction in myofiber action potential amplitude and slow rearrangement of intracellular calcium,[391] which occurs only with a change of stimulation and leads to a decreased inotropy. Such conjecture remains speculative.

B. HISTOCHEMICAL AND ANATOMICAL DEFINITION OF MYOCARDIAL PARASYMPATHETIC INNERVATION

With an overwhelming concensus from studies demonstrating a reduced inotropy in response to vagal stimulation, one must, at least, accept the results under the experimental conditions. However, anatomical and histochemical studies[34, 47, 83, 150, 175, 179, 251, 275, 276, 348, 393] have shown a high concentration of cholinergic activity within the ventricular conduction system, particularly, prior to emergence of the Purkinje fibers from the bundle branches of the conduction system.[257, 276, 348] Few parasympathetic neural branches stray from the conduction system into the surrounding myocardium; those neural branches observed traversed only a short distance.[26, 34, 47, 175, 202, 276, 291] A subendocardial plexus of neural fibers exist in the ventricles as well as in the atria.[348] Nettleship[273] showed that bilateral removal of the dorsal root ganglia in the cat caused a degeneration of the neural subendocardial plexus. Thus, histochemical and ablation studies, to date, strongly indicate that the subendocardial plexus, which shows a faint positive cholinesterase reaction,[348] probably represents an afferent network. With a very strong indication of no efferent parasympathetic fibers to the ventricles vis a vis the atrium,[47] the presence of a direct parasympathetic negative efferent effect upon ventricular inotropy, in the normally functioning mammalian heart, becomes difficult to reconcile. The overwhelming histochemical evidence that

parasympathetic postganglionic fibers exclusively innervate the conduction bundles of the ventricles[47] makes a direct parasympathetic effect upon ventricular contractility difficult to accept in the unanesthetized, non-experimental model. Proponents of a direct vagal effect upon myocardial contractility must devise a protocol that demonstrates in the unanesthetized, intact heart, probably in a well-trained dog with relatively low heart rate (unpaced between 70-80 beats per minute), that a negative inotropy precedes a reduced heart rate. In other words, proof must be offered that the resultant reduced contractility does not occur secondarily to an induced slowing of the heart, thus representing the reverse of the staircase phenomenon, or Bowditch effect. Such considerations could have important functional implications in the efferent side of the carotid sinus baroreceptor reflex. Until such a fundamental functional question gets resolved, little knowledge of the true muscarinic effect of atropine upon ventricular inotropy can be stated. With the detection of parasympathetic postganglionic fibers in the atrial musculature[47, 87, 147, 150, 175, 251] one might accept a direct negative atrial inotropy following vagal stimulation based upon the relatively large amounts of acetylcholine secreted from the atria as compared to the ventricles following vagal stimulation.[93] Should a direct negative atrial inotropy exist following vagal stimulation, then one could propose a direct muscarinic blockade of atrial inotropic reduction by atropine. The reader desiring further details should consult more extensive accounts of parasympathetic innervation of the ventricles and controls of vagal stimulation upon ventricular inotropy.[47, 175, 205, 207]

In the last section, discussion of direct muscarinic effects upon myocardial contractility, particularly ventricular inotropy, met with a reasonable degree of ambivalence. The production of negative ventricular inotropy attributed to enhanced vagal postganglionic activity becomes difficult to explain due to lack of identification of parasympathetic fiber innervation in the contracting musculature of the ventricles. Parasympathetic innervation of the ventricles does occur in the bundles of the conduction system. Much earlier in this review I drew attention to the many studies illustrating primarily the antagonist role of atropine and to a lesser extent, the agonist role of atropine upon the muscarinic receptors affecting chronotropy. Since numerous studies have shown that antagonist doses of atropine cause cardioacceleration by blocking the parasympathetic tone to the sinoatrial node, or other primary initiating foci, a unanimous consensus prevails that a direct parasympathetic control of cardiac chronotropy exists. A description of anatomical and functional characteristics related to postganglionic parasympathetic influence upon the periodicity of the heart, including the pharmacological effects of atropine, follows.

Two distinct labelling techniques have been used in late years to identify cardiac regional innervation by cholinergic fibers. The procedures rely upon the assumption that detection of choline acetyltransferase by one method and acetylcholinesterase by a histochemical technique described earlier, indicates the presence of acetylcholine which resides in the postganglionic parasympathetic cholinergic nerve fibers within the heart. The aforesaid assumption probably represents a high degree of validity, yet the reader should remember that these tests are indirect. Choline acetyltransferase occupies the cholinergic effector fibers, and the cholinesterase appears both on the fiber and at the muscarinic effector site. These enzymes contribute to the following well-known reactions:

$$\text{Acetyl CoA} + \text{choline} \xrightarrow[\text{acetyl transferase}]{\text{choline}} \text{acetylcholine} + \text{CoASH}$$

$$\text{Acetylcholine} \xrightarrow{\text{acetylcholinesterase}} \text{choline} + \text{acetic acid}$$

One major difference in the two techniques to date has been the specificity of location in the histochemical utilization of acetylcholinesterase, whereas choline acetyltransferase procedures do not offer the same degree of specificity.

Turning first to the histochemical procedures, an earlier section described their use briefly[34, 47, 83, 150, 175, 179, 251, 275, 276, 348, 393] in determining the degree of ventricular innervation by postganglionic parasympathetic fibers. These histochemical studies rely on the techniques developed by Koelle and Friedenwald[187] using acetylcholine as an indicator substrate for specific acetylcholinesterase associated with cholinergic nerve fibers.[83, 84, 189, 250, 253] Shortly after the initial study appeared, Koelle[188] published a study which outlined a differential technique for the identification of specific acetylcholinesterase in mixed tissues containing nonspecific acetylcholinesterase by using diisopropyl fluorophosphate. The concentration

of specific acetylcholinesterase appears highest in cholinergic efferents and to a lesser extent in sensory and adrenergic fibers.[190] Holmes found that nonspecific acetylcholinesterase accounted for most of the acetylcholinesterase within atrial musculature, the subendocardial neural plexus and vessel walls.[147] Only in some muscle cells and nerve bundles of the atria could specific acetylcholinesterase be found. Sinho et al.[339] found the distribution of acetylcholinesterase more plentiful in the human atrial appendage than in the canine atrial appendage. In humans the atrial appendage contained more acetylcholinesterase than the atrioventricular nodal region.[339] Human infants have higher levels (30%) of acetylcholinesterase in the atria than adults. Another major attribute of this technique has been its limited diffusion, thus adding to its location specificity.[190] The greatest intensity of staining for specific acetylcholinesterase appeared in the sinoatrial and atrioventricular nodal regions,[83, 168] with none found in association with Purkinje fibers,[168] and a very low concentration of nonspecific acetylcholinesterase was associated with nerve fibers.[168] Possibly, the small amount of nonspecific acetylcholinesterase associated with cholinergic fibers represents a precursor to the specific acetylcholinesterase of the fibers.[191] An unspecified esterase, possibly a nonspecific cholinesterase, appears associated with the M band of diaphragmatic and apical cardiac fibers[90] whereas Karnovsky[169] found nonspecific acetylcholinesterase associated with the sarcoplasmic reticulum and not M bands in functional myocardial tissue.

Two different assays for choline acetyltransferase have been developed; both require cutting, mincing and homogenization of tissue. The method developed by Hebb[139] and modified by Nordenfeldt[277] utilizes a biological assay with frog muscle. Roskowski and associates[315, 316, 317] measured choline acetyltransferase by ascertaining the amount of radiolabelled acetylcholine formation from radiolabelled acetyl CoA. Schmid et al.[330] dissected various sections of the guinea pig heart and showed by the method of radiolabelling that the sinoatrial node and base of the papillary muscle and modulator band possessed the greatest choline acetyltransferase activity while the atrioventricular node, right atrial appendage and proximal bundles had slightly less than the aforementioned areas. The ventricles contain the least amount of choline acetyltransferase activity. Roskowski and associates found more choline acetyltransferase activity in the atria than in the ventricles of chicks[318] and in the guinea pig they found more choline acetyltransferase in the right atria than in other myocardial structures;[316] however, they could not specify where in the ventricle that choline acetyltransferase resided.[317] A greater concentration of choline acetyltransferase appears in the atrial myocardium than in the ventricular myocardium of cats, dogs, rabbits and rats.[88] Lund and associates[224] found that unilateral vagatomy in the guinea pig did not appreciably affect levels of choline acetyltransferase, however, with time a slight augmentation occurred. Transplanted guinea pig heart into the abdomen of a host guinea pig showed a uniform drop in choline acetyltransferase levels in atrial and ventricular myocardium.[223] Evidently, total denervation will affect the postganglionic parasympathetic bed reducing enzyme production and thus acetylcholine formation. In the human transplanted heart, the sinoatrial node of the recipient responds to pacing and atropine, the donor sinoatrial node appears refractory to response.[33] Not only does lack of cholinergic innervation affect choline acetyltransferase levels in the region of the sinoatrial node, but the rate of production drops as neural impulses decrease.[90, 91] Following sympathectomy, choline acetyltransferase production increases.[90] Trained rats had an increase in choline acetyltransferase production in the atria (115% of control) whereas the increase in activity within ventricular musculature appeared minimal.[89] Ventricular choline acetyltransferase activity in the trained rat amounted to only about 10% of that found within the atrial myocardium.[89] Such findings reflect upon the bradycardia noted in highly trained marathon runners[289] and the bradycardia associated with myocardial infarction.[179]

Previous sections have dealt with the parasympathetic innervation of the heart and its location primarily in the right atrial myocardium and the conduction system of the atria and ventricles. Since a close association of cholinergic innervation with the structures associated with the conduction system of the heart has been established by microtechniques, and numerous studies relate to the muscarinic effects upon this system, and a plethora of work has established that atropine does temper parasympathetic influence upon chronotropy, an abbreviated review of structure-function relationships that characterize the conduction system follows. James[158, 160] and coworkers, along with many other groups previously cited, have contributed heavily to our present knowledge of the aforesaid relationships.

The sinus node of man[158] and dog[153] have very similar structural appearances. Cholinergic fibers heavily innervate the sinoauricular node,[87, 156, 175] possibly, with the greatest degree found in the heart.[34] The nerve fibers do not terminate on myofibers[158] but appear as a plexus.[275] Cholinesterase appears in all cell types within the sinus node.[150, 156, 158] Ganglia, presumably of parasympathetic origin,[150] abound in a thickly populated manner about the sinoauricular node.[151, 153, 158] Purkinje tracts originating in the sinus node form the three intranodal pathways.[155, 158] The structure of the intranodal pathways appears similar to the ventricular conduction bundles.[155] Much less cholinesterase occupies the internodal pathways than the amount found in the sinoauricular node,[158] yet ganglia and cholinergic fibers have been associated with these structures.[160] Cholinesterase levels are higher in the bundles near their junction with the atrioventricular node.[158] Three intranodal pathways augment impulse conduction from the sinoauricular node to the atrioventricular node.[155, 158] Fibers from the three intranodal pathways decussate and intermix prior to entry into the atrioventricular node[152, 155] with some entering the superior, anterior atrial border of the atrioventricular node.[152, 156] Other fibers of the intranodal pathways enter the atrioventricular nodes more posteriorly and probably represents a bypass network.[152, 158] The function of the bypass remains incompletely understood, but may allow for conduction without delay by making a detour around the area of reduced conduction. Precisely what causes the decremental conduction and where in the atrioventricular node this occurs defies appropriate explanation.[162] James and Scherf[159] have described four cell types in the A-V node. The slender transitional cells probably form the structural system responsible for conduction delay, forming a triage system which sorts and filters,[156] creating a "multiple cancellation system"[213] of sorts. Conduction delay location within the atrioventricular node remains controversial. Thaemont[355] described the posterior and lateral areas of the mouse A-V node as regions of reduced conduction, whereas James[156] and Cranefield et al.[52] in the human and rabbit, respectively, suggested that the anterior or atrial crest region performed the delay function. Thaemont[354] described cholinergic fiber innervation in the inferior section of the infant mouse atrioventricular node. The fine cholinergic fibers densely populate but do terminate[158, 175, 353] directly on cell membranes within the A-V node,[158] with ganglia of cholinergic postganglionic origin located on or about the atrioventricular node[48, 143, 150, 160] but not within the node.[158]

Innervation of the bundle of His and the bundle branches consists of postganglionic C fibers[262] which probably emanate from the atrioventricular node[348] in most species. Some earlier reports in subhuman mammals indicate the presence of ganglia in proximity to the bundles in the dog heart[276] and possibly in areas where the bundles traverse in the rabbit[34, 245] and the monkey.[245] In the human, ganglia do not appear in proximity with the ventricular conduction system.[160] The association of the autonomic system with the ventricular conduction system and the ventricles themselves has led to considerable misunderstanding, confusion and certainly a lack of clear-cut, definitive knowledge such as we presently possess for the atria and resident nodes. Using 3 day-old canine puppies, Tcheng[353] could not locate any signs of autonomic innervation in the His bundle or bundle branches using a silver reduction method for marking the cholinergic fibers. Nonidez[276] did demonstrate numerous fibers, presumably postganglionic cholinergics, in 1, 4, 7, and 40 day-old puppies. Perhaps with such few animals used by Tcheng and Nonidez, a variation in ontogenetic maturity could account for such disparity in observations.[197] Many reports do exist attesting to the appearance of cholinergic fibers in the His bundle and the proximal right and left bundle branches in humans[156, 158, 160, 175, 251] and dogs[87, 175, 276, 348] and other species.[34, 149, 150, 273] Evidence strongly supports the anatomical presence of numerous cholinergic fibers[179] in the His bundle and proximal segments of the bundle branches.[158] At the anatomical point of the proximal bundles, our precise knowledge of terminal innervation alludes us. The authors[348] identifying and tracing bundle innervation described terminal innervation as taking place when Purkinje fibers took on their characteristic, distinct, enlarged mass as compared to contractile myofibers, within the bundle branches. Nerve endings do not terminate in the bundle of His.[158] The nerve fibers test highly positive for specific cholinesterase.[149] However, we do not know if some of the fibers represent sympathetic innervation.[276] Truex and Copenhaver[360] identified nerve fibers, possibly of cholinergic origin, in intimate contact with Purkinje fibers, within the moderator band (carries a majority of the right bundle branch fibers to the free right ventricular wall); however, nerves in the moderator band identified by Stotler and

McMahon[348] apparently innervate vascular smooth muscle. Only Carbonell,[34] using the early cholinesterase test of Koelle and Friedenwald[187] (did not differentiate between specific and nonspecific acetylcholinesterase), stated that the peripheral ventricular subendocardial Purkinje plexus received cholinergic innervation. Tcheng[353] and Ehinger et al.[87] observed autonomic fibers in close proximity with the subendocardial Purkinje network. These cholinesterase-positive neural fibers probably represent an afferent sensory network[348] as nerve cells do not appear in the ventricular subendocardial plexus surroundings.[348, 393] Evidence to date precludes neural control over the subendocardial Purkinje bundle plexus[260] and its distribution of fibers to the papillary muscles and myocardial walls of the ventricles.[1] A species difference in cholinesterase content appears in the proximal bundle structures with the canine bundles containing a high content of nonspecific cholinesterase, whereas only a small quantity of nonspecific cholinesterase appears in the human.[175] Nerve fibers run parallel to the Purkinje fibers in humans,[175] sheep, oxen and pigs,[149] whereas they tend to envelop the Purkinje fibers of the dog.[176] Distal branches of the ventricular conduction system do not have autonomic innervation.[276,348]

Much of the knowledge required to understand peripheral bundle function remains unknown. Myerburg and associates[260] have shown that maximal action potential duration and maximal local refractory period occurred 2 to 3 mm prior to the entry of the Purkinje fibers into the contractile myocardium. These indices gradually increased to the stated maxima as one took measurements progressively more distal along the conduction bundles. After attaining the optimum action potential and refractory period durations, measurements progressively more distal yielded diminishing periods. At the points of maximal measurements conduction becomes minimal and Myerburg et al.[260] called this critical area the "gate." Does this area coincide with the termination of cholinergic fibers, and could muscarinic effector function at this level to reinforce the gate?

Hoffman and Cranefield[145] found that acetylcholine does not affect the depolarization of isolated Purkinje fibers, yet a later study by Baily et al.[15] has shown that, following acetylcholine, automaticity decreased and conduction increased in Tyrode solution bath at 31°C. Mubagawa and Carmeliet[256] showed that acetylcholine slowed the spontaneous depolarization of rabbit Purkinje fibers and atropine blocked the effect of acetylcholine. Possibly, muscarinic function manifests itself by throttling sympathetic response within the bundle[145] by maintaining conduction without automaticity. If a direct effect upon Purkinje fibers does not take place within the conduction bundles due to lack of a direct muscarinic effect upon Purkinje fibers[145] and the observation that nerve endings do not terminate on cell membranes in the bundle of His,[158] possibly vagal fibers influence adrenergic response. Such influence would rely upon the close proximity of cholinergic and adrenergic fibers with cholinergic excitation causing release of acetylcholine, with the subsequent short diffusion path to muscarinic receptors upon adrenergic fibers.[258] Some studies do show a conserving effect of muscarinic response upon adrenergic release of norepinephrine in the heart;[205, 214, 219, 259] however, these observations took place primarily in the atria. Functional studies have shown a more profound depressant effect produced by a vagal activity in the heart with higher levels of sympathetic activity.[205] These hypotheses, of course, would only hold true in the cardiac conduction system if adrenergic innervation occured in close proximity with muscarinic fibers within the conduction bundles. A histological trace of nerve fibers from blood vessels near the atrioventricular node entering the aforesaid node[348] has served as evidence for adrenergic innervation of the node and the contiguous conduction bundles. Many more precise anatomical studies that identify neural fiber constituents of the ventricular conduction system (differentiating sympathetic and parasympathetic innervation, if applicable), including a more definitive definition of their termination in the bundles, must take place in the future. Certainly, allied with more accurate anatomic definition, further, more discrete functional studies should pinpoint the functional interrelationships of the sympathetic, muscarinic and Purkinje systems within the conduction system, if indeed such exists. Using flourescence microsopy, Vogel and associates[378] did find sympathetic fibers running alongside the ventricular bundle branches in young calves but located essentially no adrenergic fibers within the bundle. These authors felt the contiguous adrenergic fibers did not act as effectors upon the bundle. Such studies should entail more postmortem detailed anatomic descriptions from humans and dogs with functional studies taking place in the dog.

Historically, studies[175, 348] to date have shown many anatomical similarities and few discrepancies between the two species. Only when more definitive studies take place will the desired or valid concepts describe normal conduction arrhythmic abnormalities and the muscarinic pharmacological and physiological influences upon the healthy and hypoxic conduction systems of humans at rest and during various stresses encountered in life.

XI. MUSCARINIC CONTROLS OF MYOCARDIAL CONDUCTION: ATROPINE INTERVENTION

Much of our present knowledge and understanding of the effects of atropine upon the conduction system of the heart has come from studies involving patients. Many of the patients have had cardiac disease, some of which involved the conduction system, and some had heart disease that did not affect the conduction system. Of necessity, most of the studies had poor or no design, such as associated with well-structured animal studies. However, in many instances the clinical studies that reported using atropine represent the only body of knowledge on this subject which we have at our disposal. Considering the desperate need for the information acquired and the clinicians' limited options on sick patients, in most cases, they have furnished an excellent source not only of atropine's effect upon muscarinic effectors of cardiac conduction, but, also, a better understanding of conduction function. This section summarizes the results of clinical and some animal studies detailing the conduction system function of the heart and the muscarinic affects of atropine upon the conduction system. To reorient the reader the cardiac conduction consists of the sinus node, the sinoatrial conduction pathways, the atrioventricular node, the bundle of His, the bundle branches, and the network of distal Purkinje fibers. In the normal mammalian heart depolarization commences in the sinoatrial, or SA node. A poor correlation, with an inverse relationship, exists between the sinoatrial cycle length, or heart beat, and the sinoatrial conduction time.[297] No significant correlation exists between sinoatrial cycle length and atrioventricular or distal Purkinje conduction, particularly as occurs in the bundle of His.[297] A good correlation exists between sinoatrial conduction time, A-V nodal conduction time and His bundle conduction time in human adults, as determined by multiple regression analysis.[297]

Akhtar and associates in two studies[3, 4] showed no statistical difference between ratio changes in sinus cycle length and AV conduction, when comparing responses after atropine to control. Prystowski et al.[293] found that control cardiac cycle length exceeded that observed after atropine and that propranolol augmented the reduced cycle length observed after atropine alone, thus demonstrating the role of the sympathetic system in enhancing heart rate following atropine. Atropine did not alter the ratio relationship between sinus cycle rate to atrioventricular conduction rate, indicating a relationship does exist with regards to vagal influence upon rate and A-V conduction.[3] Atropine (2 mg, intravenous) does not affect conduction in the His bundle, but it does shorten the overall conduction time.[265] Margiardia and associates[227] found that atropine caused a small though not significant reduction in atrioventricular conduction. No ganglia appear adjacent to the His bundle as they do about the atrioventricular node.[160] Such an observation has contributed to the difficulties in ascertaining the role of the muscarinic effectors in the peripheral conduction system. Administration of 0.9 mg of atropine, subcutaneously, reduced the P-R interval from 0.21-0.34 seconds to 0.04-0.16 seconds in vagotonic individuals, thus indicating a marked reduction in atrioventricular conduction time.[309]

In all individuals tested, intravenously administered atropine produces a heart rate increase proportional to the dose.[46] Patients with the sick sinus syndrome[321] will manifest a restricted response to 1-2 mg of atropine with a maximum increase to between 90-100 beats per minute.[102] Rosen et al.[313] could find no correlation between cycle length and conduction time without atropine and they did not pursue the relationship after atropine. The relationship between sinus cycle length and conduction through the AV node, His bundle and peripheral Purkinje conduction network, between individuals appears very poor, with little or no correlation.[313]

Atropine or exercise[400] can facilitate nodal conduction thereby negating A-V dissociation in some cases; first degree block and Mobitz I, or type I, second degree block respond well to atropine. A Mobitz II, or type II, second degree block does not readily respond to atropine therapy, as the origin of

this block occurs in the His-Purkinje system where direct cholinergic influence to conduction appears relatively negligible.[80, 267] The response of complete heart block to atropine seems unpredictable.[80] In complete heart block, 1.0 mg of atropine, intravenously, should be tried, nevertheless.[183] Atropine does not influence His bundle conduction in patients with complete heart block.[127] In the pentobarbital anesthetized dog, intravenous administration of atropine (40 to 700μg/kg) had no effect on the ventricular rate in the presence of complete heart block.[92] Cullis and Tribe[55] performed experiments in rabbits and cats which illustrated the absence of ventricular response following vagal stimulation after severing the His bundle. They drew two conclusions from their experiments: "(1) that all vagus fibers in passing to the ventricle run in the a.v. bundle and are therefore severed when the bundle is cut; or (2) that no fibers pass to the ventricle." Subsequently, their first conclusion became accepted as discussed earlier. When they[55] used muscarine and pilocarpine, the atria and ventricles slowed or came to a standstill; however, only the atria slowed markedly, with a slight ventricular slowing following their drugs after the bundle of His had been servered. Atropine blocked the muscarinic effects in the atria only following surgical dissociation.

One of the ongoing contemporary considerations for the clinical use of atropine involves counteracting the bradycardia which can occur following posterior myocardial infarcts.[158] This therapeutic interest arose in the late 1950's and early 1960's.[111] Still later, in the 1970's, atropine became a prominent diagnostic tool for the sick sinus syndrome[321] mentioned earlier. Atropine (2-6 mg) causes the sinus node to quicken and accelerates the A-V node.[111] Yu[398] administered 1-2 mg of atropine to patients with bradycardia in order to elevate the heart rate to around 80 beats per minute. Kimball and Kallip[183] used 1 to 15 mg of atropine, intravenously, to counter the slow sinus rhythm of 50 beats per minute or less. The onset of response took place within 2 to 3 minutes and lasted 2 to 3 hours.[183] Could this indicate a central nervous system origin for the excessive vagotonia of the sick sinus syndrome? Atropine did not elevate the heart in sick sinus syndrome[102, 313] higher than 90-100 beats per minute in a majority of cases.[321] Thus, atropine becomes a useful diagnostic tool for testing patients suspected of having sinoatrial node dysfunction.[331]

The slow heart rate that occurs following an anterior myocardial infarct in the conscious dog carries less risk for the development of arrhythmias and sudden death than do higher heart rates.[170] Atropine markedly increases the incidence of arrhythmias during or immediately after the experimental production of anterior myocardial infarct; ventricular fibrillation occurred much more frequently following atropine under the conditions set forth above.[170] Zipes and Knoebel[401] treated postmyocardial infarct patients, having bradycardia, with 0.6-1.0 mg of atropine intravenously. They observed ventricular arrhythmias for which they hypothesized may occur due to an unbalanced sympathetic activity distal to the A-V node. Lown and associates[220] have found that 0.3 mg-1.2 mg of atropine, intravenously administered, would counteract the postmyocardial infarct bradycardia for 2-4 hours. As summarized by Levitt and associates,[200] parasympathetic influence increases ventricular fibrillation threshold whereas sympathetic influence decreases ventricular fibrillation threshold in experimental ischemic preparations. Therefore, it stands to reason that a parasympatholytic agent, such as atropine, will cause a reduction in fibrillation threshold.[401] As observed by several investigators, atropine does indeed foster tachyarrhythmias up to and including fibrillation. A balancing argument for the use of atropine during bradyarrhythmias might be found in the work of Vassalle et al.[374] who demonstrated an inhibition of idioventricular foci with higher sinus rhythms. During vagal stimulation, they[374] found that blood potassium levels fell, which might suggest a role for this ion in spontaneous ventricular pacemaker activity under the stated conditions. In one study of acute myocardia infarcts, 22% of 480 patients experienced bradycardia.[327] A small percentage of patients with the bradycardia received between 0.5-1.0 mg of atropine sulfate initially as bolus intravenous injections. Eighty-eight percent of those administered atropine completely lost their premature ventricular beats and improved their systolic pressure. In 10% of the patients, ventricular tachycardia became pronounced and in one patient ventricular fibrillation ensued.[327] Those in this study who received 2.5 mg of atropine sulfate developed major adverse effects within 2-5 hours.[220] In tests run on patients, some who had heart disease, Neeld et al.[270] showed that various muscarinic blocking agents can promote response variations; that is, atropine tends to promote a greater incidence of sinus

arrhythmias, whereas methscopalamine bromide promotes a greater degree of ventricular arrhythmias. Such observations might aid in finding a more suitable analog for the treatment of postmyocardial infarct bradyarrhythmias. Certainly, use of the same anion in the salt form would facilitate the evaluation. Methscopalamine will cause tachyarrhythmias through fibrillation, just as atropine does.[249] Not only will the analog influence the response to bradyarrhythmia treatment, but the amount and periodicity of administration will also. Mogensen and Orinius[249] tried to augment a postinfarct bradycardia with a serial increment of 0.5 mg of atropine over a six hour period. A nodal bradycardia persisted until the last dose, which precipitated a tachyarrhythmia that led to ventricular fibrillation. In another study, patients administered 2 mg of atropine, intravenously, to counter a sinus bradycardia, showed an increase in heart rate from 45 beats per minute to 60 beats per minute, on an average.[268] No correlation existed between the sinus cycle length, nodal conduction and His-Purkinje conduction within this group, prior to or after atropine.[268] The only proper way to assess conduction time relationships would involve cycle frequency variation studies within single individuals in a group using graded doses of atropine.[3]

Pavlov[285] stated that 69% of the well-trained individuals he studied had a bradycardia where the conduction time (presumably atrioventricular conduction time) appeared "relatively shortened" instead of prolonged. Atropine or exercise decreased a Mobitz I block,[400] indicating that both counteract the impact of vagus tone. Does the bradycardia of exercise have a different etiology possibly akin to the sick sinus syndrome, but not observed in post-myocardial infarct patients due to the abbreviated conduction? According to Carleton et al.[35] atropine did relieve the strong parasympathetic influence on the atrium and A-V node in a well-trained athlete. Do the bradyarrhythmias of all athletes have the same etiology and respond to atropine in a like manner? Atria of exercised rats responded to atropine with a rate increase lower than that noted for atria from non-exercised rats, *in vitro*.[340] Could this point to an increased acetylcholine content in the myocardium of the trained rat? A correct explanation for the etiology of the bradyarrhythmias found in the trained athlete requires further work;[131] however, several groups of investigators[110, 210, 240] have observed a reversal in the blocks associated with the A-V node and sinus arrhythmia after administration of atropine. Thus, a body of evidence does exist for augmented vagal influence upon chronotropic relationships within the myocardium of the highly trained athlete. Possibly, sympathetic tone attenuates in the highly trained athelete for vagal stimulation of animals subjected to bilateral thoracic sympathectomy developed bradycardia and a drop in pressure. Atropine, 2.0 mg, in man increases heart rate above that seen for a given level of control exercise with a diminishing degree of augmentation to essentially no difference at marked or maximal degrees of exercise,[312] indicating that cardioinhibitory influence upon heart rate declines with ever increasing levels of physical stress.

The bradycardia which may follow a myocardial infarct will readily disappear following atropine therapy.[206, 321] The atropine (0.3-1.2 mg) effect persists for 2-4 hours.[220] The etiology of the postinfarct bradycardia may represent a pathophysiological manifestation of the Bezold-Jarisch reflex.[221] The excessive use of atropine for postmyocardial infarct bradycardia can produce an unwanted tachycardia due to too much vagolytic effect.[2, 134] Thomas and Woodgate,[356] on the other hand, found that 0.6-2.0 mg of atropine administered intravenously promoted more manageable heart rate and blood pressure levels after postmyocardial bradyarrhythmia and facilitated their patients' recovery. The administration of atropine can either exacerbate the severity and duration of pain due to myocardial ischemia, even with adequate coronary blood flow, or ameliorate the pain dependent upon the level and rate of administration.[382] Atropine-induced arrhythmias, following treatment for postmyocardial infarct bradycardia, may result from an increased loss of potassium.[234] One must remember that increased oxygen demand occurs at the time some regions experience decreased oxygen delivery, which in reality causes regional functional myocardial hypoxia.[234] These hypoxic areas then become foci of hyperirritability associated with ectopic beat formation.[306] The rapid increase in heart rate, due to a rapid increase in plasma atropine levels could cause an elevated work demand in the affected area thereby augmenting the pain associated with oxygen deprivation due to local coronary insufficiency. Pain amelioration from subcutaneous administration of 1 mg of atropine might come about by a mild increase of flow to a damaged area with little increase in work of the inflicted area. Atropine, 0.6 mg, intravenously adminis-

tered, did not alter the myocardial oxygen consumption (derived rather than measured)[98] in the anesthetized dog.[367] Moreover, Wayne and LaPlace[382] observed that 1.8 mg of atropine exacerbated the severity and duration of pain due to myocardial ischemia, even with a purported increase in coronary artery blood flow. With regards to the action of atropine upon the heart, Averill and Lamb[13] stated 3 responses may take place, depending upon the plasma levels of atropine.

With low levels, an initial vagotonic effect appears, followed by a transient period of vagal imbalance which may manifest itself at various levels of the cardiac conduction system. Usually at higher levels of atropine the attendant vagolytic responses, such as elevated heart rate and an array of arrhythmias, arise depending upon the state of the myocardium. An initial dose of intravenous atropine, 0.8-1.2 mg, can cause a transitory atrioventricular dissociation. All segments of the cardiac conduction system do not necessarily respond simultaneously to a given dose of atropine; these disparities lead to varying degress of vagotonia and vagolysis, depending upon the initial intramyocardial distribution of atropine.[13] Response disparities affecting the accustomed synchrony of the sinoatrial node and atrioventricular node may cause varying degrees of atrioventricular dissociations. In certain Wolfe-Parkinson-White syndrome cases after atropine, the reentrant phase becomes neutralized as foci nearer the A-V node conducts normally through the A-V node and the more rapid Kent bundle; however, pulses through the normal A-V node channel depolarize the muscular tissue prior to that of the faster Kent bundle.[13, 266]

The use of atropine with other drugs, such as neostigmine, can cause serious arrhythmias and conduction abnormalities.[13] However, in the case of acetylcholinesterase blockade by organophosphate poisoning, delivery of atropine must be rapid to offset the fulminating development of complete heart block, atrial standstill, idioventricular rhythm and marked hypotension.[279] Such reduction in blood pressure can take place within 1 to 3 minutes[279] following contact with toxic levels of organophosphates thus rendering intramuscular atropine administration (2.0 mg) even by the most rapid means[232, 350, 361] of doubtful therapeutic advantage following exposure to highly toxic doses of the organophosphate. High concentrations of atropine may act as a competitive inhibitor of acetylcholinesterase. Yet, at lower concentrations, atropine functions as an enzyme activator in the frog's heart.[399]

The exact influence of atropine upon peripheral Purkinje conduction awaits further studies.[94, 333] See the discussion in upcoming paragraphs on cholinergic influence within the Purkinje system. The combination of atrial pacing and atropine in heart disease can elicit an S-T segment depression on the electrocardiogram and at times angina pectoris.[329] Use of atropine in the patient with postmyocardial infarct bradycardia can lead to tachyarrhythmias, including fibrillation and also an increase in infarct size.[329] Evidently, atropine in the presence of myocardial hypoxia can lead to increased irritability of the heart. Wills et al.[388] believed that the fibrillation observed following atropine therapy for tetraethylpyrophosphate poisoning resulted from increased oxygen demand in the anoxic heart. Goldstein and coworkers[118] also found a higher incidence of occlusion and release ventricular arrhythmias in atropine treated dogs sedated with morphine and diazepam. Han et al.[132] found that ventricular ectopic foci appeared more frequently following coronary occlusion, sudden elevation of aortic pressure or hypothermia when the basic heart rate, due to vagal stimulation, became depressed in dogs. Possibly, their observations stem from a similar set of mechanisms associated with atropine, that is, augmentation of sympathetic effects, but emanate from the parasympatholytic effect of sodium pentobarbital.[215, 269] Enhanced automaticity in His-Purkinje cells can occur in the presence of catecholamines, hypoxia and stretch.[133]

The exact cause of the postmyocardial vagotonia remains unresolved.[319] Rotman et al.[319] attached some significance to the anatomical relationships, the affected large coronary vessels, location of the occlusion and the affected conduction structure to the bradyarrhythmia development. In anesthetized dogs, ischemia production increased the number of beta adrenergic receptor sites, yet the muscarinic sites still exceeded the beta adrenergic sites by 50-70%.[257] What implications their observation holds in the etiology of postmyocardial infarction bradycardia remains unknown. Webb and coworkers[83] observed that 68% of 74 patients developed overt anatomic symptoms within 30 minutes after the myocardial infarct. Vagotonia comprises over 50% of the disturbances leading to bradyarrhythmias, including heart blocks. Intravenous administration of atropine, 0.3 mg-1.2 mg, augmented the heart

rate and systolic blood pressure markedly to a mean of 93 beats per minute and 129 mmHg, respectively. However, even with care[383] 14% of the patients developed tachyarrhythmias. Atropine improved heart blocks in some patients, but not those with complete heart block.[383] When heart rate does improve in patients with heart block, following intravenous administration of atropine, the heart block probably occurs in the atrioventricular node, whereas a refractory response to atropine usually indicates the complete block occurred in the His bundle.[329] The efficacy of atropine with respect to counteracting the bradyarrhythmia following a myocardial infarct and most particularly those bradyarrhythmias associated with a complete heart block appeared much greater when administration took place within 8 hours of the infarct.[2] Success with counteracting bradyarrhythmia using atropine diminished as the duration between the attacks and the period of administration increased.[2] The reasons for this discrepancy in the antagonist action remains unsolved. Thus, besides the dose, status of myocardium and hemodynamic considerations, an etiology exists which has temporal constraints upon atropine's ability to counter the marked vagotonia associated with marked myocardial ischemia. Possibly the status of the myocardium (including the conduction system) and/or hemodynamics enters an irreversible phase with respect to muscarinic responses.

In the presence of atropine, stimulation of the vagosympathetic trunks causes a decrease in A-V conduction time, which will not occur in the presence of beta-blockade.[207] In the intact heart, paced at a constant rate, acetylcholine causes an increased atrioventricular conduction time, while atropine abbreviates the same conduction time.[207] A difference in atrioventricular conductance response after vagal stimulation during spontaneous heart rate and paced hearts occurs because of rate changes in the former which can cause indirect cycle length changes due to an indirect as opposed to the direct effect in the paced heart.[230, 231] These differences[231] cause problems in the interpretation of observations from unpaced hearts thereby accounting for the paradoxical reduction in atrioventricular conduction time during vagal stimulation in the unpaced heart. Cholinesterase concentration in the nodal tissue exceeds the concentration found in surrounding tissue by a factor of 3.[207] Cholinergic fibers richly innervate the atrioventricular node and as in other tissues, atropine will abolish or moderate atrioventricular function.[207] Unlike the A-V node, the His bundle recordings demonstrate little variation in conduction. Conduction in Purkinje fibers exceeds the rate associated with contracting myocardial cells; the reported Purkinje fiber velocity does vary from author-to-author.[328] Curtis and Travis[57] observed that Purkinje velocity varied from 3.9 to 4.5 meters/second in an *in vitro* preparation of ox conduction tissue. The greater the sympathetic activity, the more pronounced depressant effect vagal activity will have.[205] The atrioventricular node contains a rich autonomic innervation which together with the intrinsic conduction properties of the nodal cells determines the overall rate of atrioventricular node conduction.[231] Yoon and associates[397] showed that 0.5 mg/kg of atropine reduced the fibrillation threshold by 33% and that this threshold dropped markedly (approximately ⅔ of control) in the presence of ischemia in the dog. Apparently, the major influence in the profound drop in the fibrillation threshold involves myocardial ischemia, for atropine did not lower the threshold appreciably in the ischemic heart. Both propranolol and vagal stimulation promote a higher fibrillation threshold.[397] Karsh et al.[170] noted that arrhythmias occurred at a greater rate upon occluding a coronary artery in atropine-treated animals. Occlusion release arrhythmias and suddent death by ventricular fibrillation occurred more frequently in the atropinized dog. Sympathetic activity tempers the vagal influence upon heart rate control.[381]

From studies in laboratory animals and human beings, it appears that the intrinsic control systems are adequate for basal cardiac function and for responses to moderate stress, including low levels of exercise; however, extrinsic control systems (the autonomics) must function well for severe stress situations in order to insure an adequate, timely response.[178] The hypothalamus influences the sympathetic (posterior hypothalamus) and parasympathetic (anterior hypothalamus) preganglionic axons of the medulla (parasympathetic) and spinal chain (sympathetic).[114] Heart rate changes due to neural influence, result from a reciprocal innervation of the autonomic nervous system.[314]

Warner and Russell[381] derived the following equation from combined vagus and sympathetic nerve stimulation, thus describing the resultant heart rate:

$HR_{SV} = HR_V + (HR_S - HR_0)(HR_V - HR_{min}/(HR_0 - HR_{min})$

S = sympathetic 0 = no stimulation

V = vagal min = minimum heart rate by vagal stimulation alone short of standstill

This equation does impart a predominant influence to the vagus in control of the heart rate as compared with the sympathetic input. Using a different approach than Warner and Russell,[381] Cavero and coworkers[40] established their estimate of the autonomic nervous system upon myocardial chronotropy. To understand the involved autonomic elements functionally, they studied heart rate in the unanesthetized dog following muscarinic blockade with methylatropine (HR_A), beta-adrenergic blockade with either propranolol or practolol (HR_p), or total simultaneous blockade with the above agents (HR_0). From these studies Cavero et al.[40] derived the following mathematical relationship to describe resting heart rate (HR_N):

$HR_N = HR_0 \times HR_A/HR_0 \times HP_P/HR_0 \times W$

W = parasympathetic-sympathetic interaction equal approximately to 1

From these studies these investigators[40] came to a conclusion similar to that of Warner and Russell,[381] namely, that parasympathetic tone predominates over sympathetic tone in moderating of the resting heart rate of the dog. The interactions of the autonomic system innervating the heart do not exist as simple summed algebraic relationships, but rather as complex interactions which may involve the monophosphates of adenosine and guanosine.[204, 206]

Zipes and Knoebel[401] observed an increase in paroxysmal ventricular complexes in 2 patients post-myocardial infarct, following intravenous administration of atropine. Atropine (0.5-1.0 mg) reduces A-V conduction time, but not atrial conduction time; yet, the effective refractory periods remained essentially unchanged.[27] Bissett et al.[27] found a significant reduction in the nodal functional refractory period following administration of atropine. Yarullin and coworkers[396] observed, in a majority of humans studied, that slowing of rhythm did not change atrioventricular conduction in a majority of subjects; however, in approximately 37% of subjects studied conduction increased. These authors felt that the divergence of response might take place through a disparate muscarinic effect upon the right or left vagus. Possibly, more atropine enters the central nervous system in some individuals, thereby accounting for a differential in response. The ultimate answer will await futher controlled experiments. Atropine caused a migration of the pacemaker within the sinoauricular node, to the coronary sinus, to the atrioventricular node and dissociation concomitantly with atrioventricular interference leading to the development of ventricular extrasystoles. Patients with autonomic vascular dysfunctions developed more frequent bouts of arrhythmias following administration of atropine.[396] All arrhythmias attributed to the coronary sinus probably arise from the atrioventricular node.[160]

At heart rates between 50 and 90 beats per minute, the ventricular fibrillation threshold diminished with reduced vagal tone.[179] When edrophonium blocked acetylcholinesterase activity, the fibrillation threshold increased in ischemic and non-ischemic hearts.[179] The protective effects of cholinergic stimulation becomes more obvious in the presence of adrenergic tone.[179] Muscarinic agents do increase ventricular fibrillation threshold as established by tests utilizing repetitive extrasystoles; atropine serves to nullify the muscarinic response[296] and reduce it below baseline control values.[296] In the anesthetized dog, Harrison and coworkers[137] showed that acetylcholinesterase inhibition or vagal stimulation raises the fibrillation threshold 145%. Atropine does not significantly reduce the fibrillation threshold when used alone; however, in the presence of left anterior descendens occlusion, atropine did lower the ventricular fibrillation threshold.[137] The studies accomplished by Harrison et al. took place with either light sedation with diazepam and morphine or chloralose anesthesia. However, the incidence of release-of-occlusion arrhythmia increased following the administration of atropine in dogs sedated with morphine and diazepam.[118] Atropine caused an elevation in heart rate in the conscious dog with a coronary occlusion; the S-T segment significantly ($p < .01$) and concomitantly elevated with heart rate elevation ($r = .81$); the increased injury solely appeared rate mediated.[304] Healthy humans devoid of heart disease showed an elevation in fibrillation threshold upon elevation of heart rate with an increase in

refractory period. However, in patients with myocardial infarcts, the opposite responses occurred.[176, 177, 225] In cats anesthetized with chloralose, administration of atropine (1 mg/kg) or vagotomy following coronary occlusion caused a highly significant percentage of deaths.[38] Electrical pacing did not produce the same high rate of deaths after coronary occlusion yet did not reduce the fibrillation threshold.[38] Cats having an intact vagal innervation did not experience a high rate of post occulsion arrhythmia; however, following administration of atropine, the incidence of arrhythmias increased.[38] In the absence of ischemic conditions in the dog under pentobarbital anesthesia, atropine did not cause any consistent fibrillation threshold response.[38] Evidently, the status of the autonomic pathways and the integrity of oxygen delivery within the heart determines, to a large extent, the effects of atropine upon the ventricular fibrillation threshold. Presence of an active sympathetic tone will insure a vagal nerve stimulation-induced elevation in ventricular fibrillation threshold.[192, 205]

In morphine-pentobarbital anesthetized dogs administered calcium intravenously, Page et al.[284] found that intravenous administration of atropine (1.0 mg/kg) augmented ventricular ectopic activity which led to ventricular tachycardia and ultimately fibrillation. Evidently, the combination of calcium and atropine lowers the fibrillation threshold markedly. Atropine caused ventricular ectopic beats which became more prevalent as the dose level of pentobarbital decreased; this response occurred due to the muscarinic blocking action of atropine either directly upon the musculature or to the resultant tachycardia.[294] Increased sympathetic activity promotes ectopic foci development.[133] The risk of fibrillation increases when ectopic beats become closely coupled and fall in a vulnerable period.[133] The influence of ischemic areas upon depolarization and repolarization wave fronts, causing their fractionization and reentry, should enhance fibrillation.[133]

Usually, the sinoatrial node pacemaker activity reacts more readily to autonomic stimulation than the atrioventricular node.[344] We possess very little knowledge about the neural influences upon atrial intranodal fibers;[160] hence, our understanding of the actions of atropine upon these fibers will require further study. The state of the heart does determine its sensitivity to autonomic stimulation; this may include not only nutrition, ionic balances and the level of oxygenation, but also rhythmicity. Rhythmicity could affect the functional refractory period of the A-V node. Phasic vagal activity can cause first degree and type I second degree heart block.[344] Interestingly, following atrioventricular block with formalin in the dog, Eliakim et al.[92] showed that atropine had no effect upon ventricular rate. In sedated man paced at 120 to 140 beats per minute from the right atrium, Carleton and associates[36] found that the P-R interval increased with an increased heart rate; however, atropine significantly ($p < .005$) shortened the P-R interval for any given heart rate. The authors claim that this confirms previous findings which associate a prolonged atrioventricular conduction with an elevated atrial heart rate. Such runs counter to subsequent work by Akhtar and coworkers[3, 4] described earlier in this review, and would appear to run counter to our present understanding of vagal influence upon the atrioventricular node. These patients[36] received meperidine and sodium pentobarbital prior to the study for purposes of sedation. Could these observations[36] represent a unique train of events due to the sedating drugs and/or right atrial pacing, per se? Possibly, atrial pacing stimulates indigenous cardiac ganglia or some combination of effects with pentobarbital. Even with atropine, the P-R interval increased with heart rate, although at a lower level.[36] Stimulation of the vagus caused the usual block and conduction delays at the A-V node in anesthetized cats — at elevated voltages heart rate increased with a concomitant reduction in atrioventricular conduction.[285]

The effective refractory period of the atrioventricular node decreased as the heart rate increased during exercise or after the administration of atropine.[246] Prystowski et al.[293] showed that atropine attenuates the effective and functional refractory periods of the ventricle and most likely the atrioventricular node of humans. Evidently, vagal stimulation, or acetylcholine, does not affect ventricular repolarization but apparently hastens the repolarization of atrial fibers.[144] This observation would indicate a probable increase in atrial repolarization due to atropine, such a probability awaits the proper experimental evidence for true substantiation. Atropine did not cause a change in the relative refractory periods of the major ventricular bundles and the Purkinje system.[27] Using a fixed heart rate, Bissett et al.[27] found a slight but not significant change in the effective refractory period of the A-V node; however, the

functional refractory period of the A-V node did show a significant reduction ($p < .005$) in the human heart. Atropine did not affect the effective refractory period of human atrial tissue, but it did alter the pattern of conduction of atrial premature beats.[27] Interestingly, the loci of stimulation did affect the length of the ventricular effective refractory period with a longer refractory period with right atrial pacing than with right ventricular apex pacing.[128] The ventricular effective refractory period duration showed basic cycle length dependency[128] and the effective refractory period varied somewhat from day-to-day. Guss et al.[128] found that 1 mg of atropine did not produce significant nor consistent changes in the effective refractory period in humans.

In studies of humans that have paroxysmal supraventricular tachycardia, two A-V nodal pathways for impulse transmission exist as outlined by Wu et al.[394] Antegrade conduction took place via a slow pathway and retrograde conduction took place via a fast pathway. Atropine[394] accelerated conduction via both pathways and in effect shortened the cycle length. Atropine shortened the fast pathway effective refractory period significantly to 88 $\pm$ 3% SEM of control but did not significantly influence the antegrade effective refractory period. The meaning for this divergent observation remains unclear, except that the effective refractory period appears longer in the fast pathway.[328] In unsedated humans paced at 150 beats per minute, intravenous atropine alone, or in combination with intravenous propranolol caused a significant reduction in ventricular effective and functional recovery periods.[293] Atropine alone promoted a significantly greater reduction in the ventricular effective recovery period, than it did in combination with beta adrenergic agent; however, the differences observed for the functional recovery comparing atropine alone with the combination did not appear significant.[293] Possibly, the latter finding might stem from too few samples. In pentobarbital anesthetized open-chested cats, 0.2 mg/kg of atropine sulfate, administered intravenously, caused a slight but significant increase in the left ventricular refractory period.[28] This represented the opposite from that expected, the investigators[28] ascribed the extended ventricular functional refractory period[324] to blockade by atropine of muscarinic effectors in the sympathetic ganglia because cardiac sympathectomy negated augmentation.

In dogs anesthetized by using chloralose with atrial pacing at 120 and 200 beats per minute, Martin and Zipes[233] increased the effective refractory period of ventricular epicardium, endocardium and septum. Physostigmine potentiated the vagal response, as did propranolol. Vagal stimulation, in the presence of atropine, not only blocked the prolongation of the ventricular effective refractory period, but also actually caused a slight reduction in the effective refractory period, probably due to sympathetic influences of unknown etiology.[233] Valora et al.[364] produced a transient, but pronounced increase in the T wave amplitude, following an intravenous infusion of atropine (36μg/kg), thus adding credence to the possibility of cardiac repolarization changes due to this drug.

Das and coworkers[67] found that intravenous doses of atropine in humans, ranging from 0.1 mg to 0.3 mg, caused a slight reduction in heart rate and in atrioventricular node conduction time. At 0.4 mg of atropine, heart rate remained essentially the same; however, the atrioventricular conduction time declined to 89% of baseline. Atropine shortens or quickens impulse transmission through the atrioventricular node and may equalize or shorten the conduction time through the normal pathway as opposed to the auxillary pathway in the Wolf-Parkinson-White syndrome.[266] At 0.8 mg of atropine, heart rate increased by 30%, and the atrioventricular conduction time diminished by 9%.[67] No correlation existed between sinus nodal rate and atrioventricular conduction. James[157] found that 10 μg/ml of atropine injected directly into the SA node artery blocked the muscarinic effects on the sinoatrial node, but did not stimulate adrenergic response. In patients with severe yet unspecified heart disease, .04 mg/kg of atropine did not produce the customarily large heart rate augmentation[164] usually observed. Cholinesterase inhibitors selectively perfused into the atrioventricular node artery produced A-V nodal block which atropine counteracted.[207]

In the heart devoid of disease, vagal tone causes greater influence upon sinus cycle length due to the greater sensitivity of the sinus node in comparison with the atrioventricular node.[344] Changes in the fundamental rhythm of the heart or physiological state of the heart[154] after exposure to autonomic influences, particularly at the A-V node, probably takes place by entry during the functional refractory period.[344] As discussed earlier, vagal activity can produce delay patterns resembling first degree and/or

Mobitz I type blocks in the atrioventricular node.[344]

A group of patients, with a history of different cardiac diseases, underwent electrophysiological evaluation for atrial and sinoatrial conduction 5 to 10 minutes after an intravenous injection of atropine sulfate.[199] Atropine did not significantly affect interatrial conduction time nor right intraatrial conduction time when all subjects were compared or when those with resting heart rates greater than 60 beats per minute were compared. Those patients with a control resting heart rate less than 60 beats per minute had a right intraatrial conduction time of significantly ($p < .025$) less duration following atropine. Due probably to group size, sinoatrial recovery time corrected for heart rate, did show a significant reduction after atropine.[199] However, upon evaluating this group after dividing them by those having resting control heart rates above or below 60 beats per minute, an estimate of statistical insignificance occurred for both groups. Further studies of both groups must take place to promote a reasonable understanding of the effect of atropine upon atrial repolarization. The present study[199] represents a heterogeneous population, with several types of heart disease, rendering one evaluation rather tenuous. Atropine significantly reduced the basic cycle length and sinoatrial conduction time in all three groups mentioned above. Possibly, atropine acceleration of the sinoatrial conduction time indicates that the intranodal pathways do fall under vagal influence.[199] More studies must take place to verify the degree of vagal influence upon atrial internodal pathways. However, very little correlation exists in *in vitro* testing between basic cycle length and atrial conduction times. A poor positive correlation exists between the basic cycle length and the reduction in action potential shortening of rabbit atrial musculature.[241]

Han[133] showed that a highly significant positive correlation exists between cycle length and action potential duration. Also, a highly significant positive correlation exists between the degree of ectopic beat formation and the length of cycle in dogs with coronary occlusion[133] under pentobarbital anesthesia.[269]

Danilo and associates[65] induced a dose-related reduction in canine Purkinje fiber spontaneous rate using acetylcholine in a Tyrode bath at 37.5°C. This effect represented a confirmation of the decrease in spontaneous depolarization of phase 4 of the Purkinje action potential.[145] Atropine (2×10^{-6}M) blocked the graded responses to acetylcholine. In an extension of the above work, these workers[65] found that ventricular rhythms from their blocked hearts probably originated in the more distal conduction bundle due to extensive damage to the atrioventricular node and bundle of His due to formaldehyde infiltration. Atropine in the presence of propranolol and acetylcholine increased the idioventricular rate, thereby suggesting a muscarinic slowing of the peripheral conduction system's spontaneous rhythm.[145] In patients with known complete heart block and dissociation, intramuscular administration of atropine will increase the auricular rate to a greater extent; however, the ventricular rate augments somewhat and overrides ventricular ectopic foci.[332] Even though the inherent rate of ventricular depolarization lags behind the characteristic rates of the atrium, these studies appear to suggest some muscarinic effect over ventricular depolarization.[332] Narula and Narula,[264] using atropine, showed that when a heart block occurred proximal to the His bundle, a significant change in ventricular heart rate occurred; however, when the block occurred within the His bundle, an insignificant heart rate change took place. In light of James' study[158] implicating the occurrence of conduction slow-down to the atrial part of the atrioventricular node, possibly the muscarinic receptors reside in that region, also. Such reasoning remains tenuous, for positive identification of cholinesterase appears throughout the node and further into the bundle of His.[158] Secondly, the precise location and actual mechanism of A-V delay remains unknown.[162] In studies of acetylcholine function in the A-V node, Cranefield et al.[52] showed that impulse transmission failure took place at the atrial margin of the node. Acetylcholine depressed depolarization (phase 4 of the action potential) and reduced the action potential amplitude; their major observation was the fragmentation of the action potential.[52] Martin,[231] in a review, has brought forth the controversy of just where in the node delay occurs and suggests, as James has, that this delay function requires further study. Pacemaker fibers within the His bundle do not appear to respond to muscarinic influence to a great extent, as illustrated by the lack of profound response following atropine.[264] In the dog anesthetized with sodium pentobarbital, infusion of acetylcholine (0.1 μg, 1.0 μg or 10 μg) into the septal artery blocked atrioventricular conduction.[161] In the same type preparation, infusion via the A-V node artery

of 10 μg/ml of neostigmine produced heart blocks of varying degrees to complete heart block within 6-10 minutes. Atropine, 10 μg per ml, reversed the response to neostigmine.[161] Intravenous infusion of 0.5μg/kg and 20μg of atropine into the artery supplying either the sinoatrial node or the atrioventricular node produced a complete muscarinic blockade of the appropriately treated node.[158, 402] Recent studies by Loeb and associates[218] suggested that the muscarinic receptors in the sinoatrial node and atrioventricular node may differ functionally. They based their hypothesis upon the observations that with continued vagal stimulation, cycle length prolongation dissipated with time; however, atrioventricular conduction remained prolonged throughout the period of stimulation. A highly significant correlation ($r = .94$, $p < .001$) existed between the stable rates of the SA node and AV node, with the automaticity of the atrioventricular node representing approximately ⅔ of the sinoatrial node in the anesthetized open-chested dog.[363]

Humans with hyperthyroidism respond to atropine in a manner similar to the euthyroid.[140] Hyperthyroid patients will exhibit an initial bradycardia followed by the characteristic tachycardia associated with atropine. However, those persons afflicted with overactive thyroids showed a 22 ± 2 SEM percent elevation of heart rate whereas euthyroids demonstrated a 38 ± 5 SEM percent change — this difference showed significance at the .005 level. The reason for this difference in elevation stemmed from the initially higher heart rates observed in hyperthyroid patients. The attained heart rate levels following the same dose of atropine did not show a statistical difference between the 2 groups. Possibly, hyperthyroidism may cause a reduced vagal influence over control heart rate[140] or the increased metabolic rate could promote a greater resting oxygen demand causing chemoreceptor mediated heart rate augmentation.

XII. A POTENTIAL FOR MUSCARINIC MODULATION OF NOREPINEPHRINE OUTPUT

Earlier in this review, there appeared reference to a possible mechanism for muscarinic modification of sympathetic influence upon the myocardial conduction system. The proposal for an association of muscarinic receptors at the synaptic end of sympathetic postganglionic fibers first appeared in an article written by Burn and Rand.[32] Lindmar and associates,[214] using a Langendorf rabbit heart preparation, demonstrated the possible presence of muscarinic receptors on peripheral sympathetic nerve endings by perfusion of small doses of acetylcholine with the subsequent reduction of norepinephrine output. Increasing doses of atropine from 10^{-9} to 10^{-6} g/ml increased the output of noradrenaline in the presence of acetylcholine in a dose related manner.[214] Doses larger than 10^{-6} g/ml of atropine depressed noradrenaline output.[214] Also, 10^{-5} g/ml of 1, 1 dimethyl-4-phenylpiperazinium iodide (DMP),[43] a ganglionic stimulating agent, in the presence of increasing amounts of atropine up to 10^{-6} g/ml, did not affect the amount of norepinephrine output. Greater doses of atropine in the presence of DMP did cause a reduction in norepinephrine output. From these experiments, Lindmar and coworkers[214] had hypothesized a muscarinic control of norepinephrine with the muscarinic receptors on the postganglionic sympathetic fibers. In an isolated rabbit atrial preparation stimulation of the parasympathetic system with simultaneous sympathetic stimulation caused a reduced output of norepinephrine. Atropine blocked the parasympathetic throttling of norepinephrine.[219] The reduction in norepinephrine effluent probably occurs by either recruitment of terminal parasympathetic fibers or hyperpolarization of the terminal sympathetic fiber.[259] Atropine not only blocks the muscarinic throttling of norepinephrine release in the heart, but also promotes nicotinic release of norepinephrine.[259] Muscholl[258] found that inhibition of norepinephrine release starts at 5.5×10^{-8} M and reaches its maximum at 10^{-6} M acetylcholine. Cholinesterase inhibitors will enhance the throttling response.[258] Using a similar cat Langendorf preparation as Muscholl's group did, Haeusler et al.[130] found that acetylcholine ($3.4 \times 10^{-7} - 5.5 \times 10^{-7}$ M) increased the output of norepinephrine. Haeusler's group[130] recognized that their results differed from previous work by Lindmar and coworkers;[214] they offered no equitable explanation for the differences. At that time a species difference could have offered a suitable explanation; however, subsequently, muscarinic doses of acetylcholine have been found to reduce norepinephrine release in the cat,[258] which reversed in the presence of atropine. Acetylcholine may have both presynaptic and postsynaptic inhibiting

affects upon sympathetic stimulation in the heart and may exhibit exaggerated antagonism in heart tissue.[116] Acetylcholine (10^{-3} M) and methacholine prevented the release of radiolabelled norepinephrine by nicotinic stimulation in the guinea pig heart.[386] Atropine (10^{-5} M) facilitated norepinephrine release inhibited by acetylcholine (10^{-6} M).[386] In the presence of sympathetic stimulation, vagus stimulation resulted in a frequency related attenuation in coronary sinus catecholamine concentration.[198] Muscarinic inhibition of endogenous myocardial catecholamine liberation took place in the dog.[198] The reduction of dp/dt and heart rate correlated well with the reduction of catecholamines in the coronary sinus blood.[198] Atropine blocked some of the throttling effects of vagal stimulation, showing a greater effect against 4 Hz vagal stimulation than against 1 Hz vagal stimulation. Although recent studies have pointed towards cholinergic modification of ventricular function, the question of whether the cholinergic influence represents a direct or indirect effect remains unsettled.[387, 403]

Not only has the modulation of adrenergic function by muscarinic mechanisms been looked at in the heart, but more recently Vanhoutte and associates have studied the effects in various peripheral arteries and veins.[366, 367, 368, 371, 372] In an *in vitro* study, electrical stimulation of canine saphenous veins produced an increased release of radiolabelled norepinephrine which acetylcholine reduced.[364] However, the reduction of norepinephrine took place only during electrical stimulation of the saphenous vein strip.[366] Neither hexamethonium nor beta blockade affected the reduction of tension due to acetylcholine in the presence of electrical stimulation.[367] Atropine (10^{-8} g/ml) blocked the reduction in tension development and norepinephrine release by acetylcholine (5×10^{-8} g/ml).[367] The degree of tension reduction during electrical stimulation of the saphenous vein appeared dose related[367] to the amount of acetylcholine administered. However, Vanhoutte and Shepherd[367] found that acetylcholine caused increased tension development due to the addition of norepinephrine in the absence of electrical stimulation. Hexamethonium did not alter the affect of acetylcholine upon tension development and norepinephrine release reductions in the presence of saphenous vein electrical stimulation, thus precluding an alteration in nicotinic activity in their *in vitro* model. Atropine (10^{-8} g/ml) blocked the acetylcholine (5×10^{-8} g/ml) induced attenuation of tension development and norepinephrine discharge following electrical stimulation of the saphenous vein.[367] This latter observation lends further credence to a potential muscarinic buffer control of sympathetic discharge and tension development in the saphenous vein, at least under the *in vitro* conditions in a controlled bath environment.[367] Further studies by Vanhoutte[368] showed that following electrical stimulation of the saphenous vein, femoral vein, anterior tibial artery, superior mesenteric artery, pulmonary artery, femoral artery or gracilis muscle arteries *in vitro*, acetylcholine reduced tension; however, in the pulmonary vein or mesenteric vein, acetylcholine, under the identical conditions, promoted an increased tension. As observed in the case of the saphenous vein, in an earlier study,[367] atropine reduced the buffering response to all other arteries and veins tested, as stated in the previous sentence.[368] Allen et al.[7] observed that low concentrations of acetylcholine (4×10^{-11} - 1×10^{-10} M) increased vasoconstriction in the stimulated isolated rabbit ear, whereas greater concentrations of acetylcholine (4×10^{-6} M) reduced the tension, as previously reported. Low concentrations of acetylcholine increased the norepinephrine release, while greater concentrations of acetylcholine (3×10^{-6} M) reduced the efflux of norepinephrine.[7] Interestingly, neither atropine nor hexamethonium affects the augmented action of low concentrations of acetylcholine in the rabbit ear artery preparation in the inhibitory throttling response fostered by acetylcholine; atropine did function as a muscarinic blocking agent.[7] Acetylcholine inhibited norepineprine effluent promoted by introduction of K^+, in the same manner as experienced during sympathetic nerve stimulation.[369] The observed reaction to acetylcholine probably demonstrated that hyperpolarization of the distal postganglionic sympathetic fiber could cause a selective change in calcium permeability.[369] Thus, Muscholl[259] and Vanhoutte and Verbeuren[369] both suggested hyperpolarization as a possible reason for the throttling effect of acetylcholine upon norepinephrine release and function. In other words, acetylcholine blocks depolarization of the sympathetic fiber, thus attenuating adrenergic transmission.[370] Phentolamine did not affect the levels of norepinephrine observed after electrical stimulation of vein strips *in vitro;* however, the tension of the vein strip diminished.[370] Vanhoutte and Verbeuren[370] noted that acetylcholine did not affect tension, but reduced the efflux of norepinephrine. This latter observation probably lends some credence to the existence of

muscarinic receptors on or about the presynaptic sympathetic postganglionic fiber. Inhibition of acetylcholinesterase augments the acetylcholine suppression of norepinephrine.[371] We require further histochemical evidence for the presence of acetylcholinesterase not only in association with peripheral sympathetic innervation, but also with the sympathetic innervation accompanying the conduction bundles of the heart in the absence of accompanying parasympathetic fibers.[347, 387] As stated in an earlier review by Westfall,[387] there remains further work to prove, beyond a doubt, that prejunctional muscarinic receptors do function to moderate the release of norepinephrine at the sympathetic postganglionic fiber synapse. In certain instances, such as in the sinoatrial node, the functioning of contiguous fibers from both autonomic systems could provoke the modulation of the sympathetics by the parasympathetics.[387] Vanhoutte and Levy[373] stated that cardiac inotropy and chronotropy respond to vagal inhibition of sympathetic tone by the buffering system discussed in this section. Earlier, a discussion of direct effects upon inotropy indicated doubts about the direct effects of the parasympathetics upon ventricular inotropy. Such a mechanism as proposed by Vanhoutte and Levy[373] could prove valid; however, further evidence for the parasympathetic innervation of the ventricle or the functional mechanisms of muscarinic receptors and their histological and/or molecular significance on the ventricular sympathetic fibers awaits future definitive studies. For further information on this subject, the reader should look at the reviews of Westfall,[387] Fozzard,[108] Vanhoutte,[371] and Story et al.[347]

XIII. CHEMISTRY AND STRUCTURE-FUNCTION CONSIDERATIONS OF ATROPINE

Convention dictates that whenever an author writes a review about a drug or group of drugs the initial step requires elaboration of the chemical structure and a detailed summary of the chemical characteristics. Since this review restricts itself to one of a group of drugs upon one organ system, this writer opted to plunge directly into the effects of atropine upon the muscarinic effectors of the cardiovascular system. However, upon reading many basic and clinical studies, several points became readily apparent: A consistent usage and/or comparison of dose form, i.e., salt versus the base form, lacks uniformity. Particularly at agonist levels of usage (below 0.5 mg intravenous administration per individual), no overt consideration to the age or stability of atropine in solution appears in the studies followed and these studies have produced perplexing results most prevalently following their use as a counteractant to the postmyocardial infarct bradycardia. Probably, due to the longevity of the use of this drug in modern medicine and in the laboratory and the fact that the basis for the bulk of our chemical knowledge stems from the period of Ladenberg[195] in 1881 through the 1920's, proper attention to the chemical properties of atropine has been overlooked in more recent studies.

In recent years a majority of the reported studies, where stated specifically, utilized the sulfate salt of atropine. A modification of the structural formula of atropine sulfate, as entered in *The United States Dispensary*[282] follows (Figure 1):

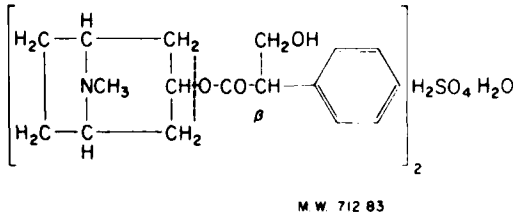

Figure 1. *Atropine Sulfate.* Tropine appears to the left of the hatched bar and tropic acid appears to the right. M.W. = molecular weight.

Atropine sulfate usually has one water of hydration.[282] Atropine represents an ester comprised of tropine (to the left of the dotted line), an amino-alcohol, and tropic acid (to the right of the dotted line).[349] Atropine possesses stereoisomerism, which Fodor et al.[106] confirmed by the methyl alcohol group's position about the ß carbon of tropic acid. Atropine represents a racemic mixture of two isomers in equal proportions,[349] namely, l-hyoscyamine and d-hyoscyamine.[58, 76] Tropic acid, per se, had no effect upon cerebral function; the response of tropine appeared considerably less than atropine; however,

tropic acid enhanced the response of tropine by 200 x's.[226] The activity and toxicity of the atropine-hyoscyamine molecule appears markedly enhanced (up to 600 times) by the methyl hydroxy group attached to the tropic acid moiety.[60] Substitution of the CH_2OH group by a methyl group on the beta carbon reduces atropine action at the myoneural junction by 99.5%.[60] Fodor and Csepreghy,[105] using compounds of known optical configuration, established the correct configuration for l-tropic acid. Studies by Lands[197] suggested that the methyl piperidyl ring of the tropine part of the molecule possessed the pharmacologically active site of hyoscyamine. The naturally occurring geometric configuration of the tropine moiety appears as a chair rather than as a boat configuration (Fig. 2), giving structural prominence to the trivalent nitrogen with pharmacological and physiological significance.[104] The steric configurations of the atropine structure referred to in the last sentence appears below:

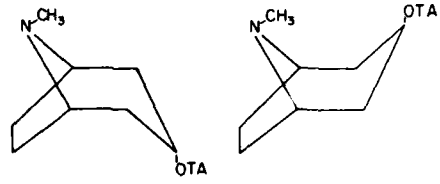

Figure 2. *Atropine Sulfate.* Steric configurations of tropine designated as chair and boat. OTA represents tropic acid.

Evidently, stereoisomerism of the tropic acid part of the hyoscyamine ester, which determines whether the hyoscyamine ester possesses dextro or levorotatory isomerism, determines the degree of receptor affinity[19] and therefore, the magnitude of antimuscarinic response, whereas the tropine moiety, the methyl piperidine part in particular, in the chair configuration manifests the focal point of pharmacological response.[197] Now that structurally functional portions of hyoscyamine have been defined, I wish to present in tabular form (Table 1), the comparative response strengths of l-hyoscyamine, d-hyoscyamine, and dl-hyoscyamine (atropine) as furnished in published experiments.

TABLE 1. Comparative Response to Atropine and Its Isomers

Reference	Comparative Response	Species and/or Organ
Cushny R[58]	l-Hyoscyamine = Atropine	Frog
	l-Hyoscyamine = Atropine	Mouse
	l-Hyoscyamine > Atropine	Pupil cat
	l-Hyoscyamine 2 x's > Atropine	Vagal block
	l-Hyoscyamine 14 x's d-Hyoscyamine	Arresting canine salivation
	l-Hyoscyamine 14 x's > d-Hyoscyamine	Heart rate response in cat
Graham DPD and JA Gunn[123]	l-Hyoscyamine 2.5 x's > Atropine	Rabbit smooth intestinal muscle
	l-Hyoscyamine 2.5 x's > Atropine	Guinea pig intestine
Cushny AR[59]	Atropine 20 x's > d-Hyoscyamine	Saliva reduction and heart rate
	l-Hyoscyamine 40 x's > d-Hyoscyamine	Saliva reduction and heart rate
Macht DI[126]	l-Hyoscyamine > Atropine	Cerebrum
Oettinger van WF and IH Marshall[260]	Atropine 31 x's > d-Hyoscyamine	Isolated rabbit intestine
	l-Hyoscyamine 50 x's > d-Hyoscyamine	Vagus to heart
	l-Hyoscyamine = Atropine	Vagus to heart
Pilcher JD[268]	l-Hyoscyamine 2 x's > Atropine	Infants and 3-4 year old children
	l-Hyoscyamine 20-40 x's > d-Hyoscyamine	Infants and 3-4 year old children

continued next page

TABLE 1. Comparative Response to Atropine and Its Isomers *(continued)*

Reference	Comparative Response	Species and/or Organ
Buckett WR and CG Haining[31]	l-Hyoscyamine 29 x's > d-Hyoscyamine	Peripheral muscarinic blocking agent
Schauman[326]	At low concentrations l-Hyoscyamine > d-Hyoscyamine difference diminishes as doses increase	Guinea pig ileum
Levy[201]	Atropine 2.1 x's > d-Hyoscyamine chloride, Atropine sulfate = d-Hyoscyamine sulfate	Frog pupillary diameter
Domino EF and RD Hudson[77]	Hyoscyamine 50 x's > d-Hyoscyamine	In dog heart rate, EEG arousal
	l-Hyoscyamine 8 x's > d-Hyoscyamine with .2% l-Hyoscyamine	EEG arousal
	l-Hyoscyamine 16 x's > d-Hyoscyamine with .2% l-Hyoscyamine	Heart rate elevation

A survey of Table 1 reveals that generally, the muscarinic antagonist response of l-hyoscyamine exceeds atropine by a factor of 2, yet atropine generates approximately 25 times the response of d-hyoscyamine, while l-hyoscyamine shows a potency slightly less than 30 times d-hyoscyamine. Lieber and LeBarre[211] showed that l-hyoscyamine possessed twice the toxicity of atropine sulfate based upon LD_{50} tests in guinea pigs. The results of Levy[201] indicate that the salt form does affect the degree of response to atropine in the frog with the chloride salt of hyoscyamine promoting less pupillary dilation than the sulfate salt. Schauman[326] demonstrated that the hyoscyamine isomers possess a concentration of maximum response which can ultimately lead to equal molar response of d- and l-hyoscyamine which would ordinarily demonstrate great differences in their ED_{50}.

Many authors, beginning with Ladenberg[195] have described the isomers of hyoscyamine. Products from the synthesis of atropine in the laboratory and the extraction of l-hyoscyamine from plants, such as *Atropa belladonna,*[349] followed by purification processes,[146] show the same elevation in heart rate.[196]

Specific standards for optical rotations of hyoscyamine isomers have been established. Levo-hyoscyamine has a specific rotation of $-21°$; dextro-hyoscyamine has a specific rotation of $+21°$ in dilute ethyl alcohol and the specific rotation of atropine equals 0°.[37, 146, 244] The standard specific rotation for the most widely used isomer of hyoscyamine, dl-hyoscyamine sulfate, or atropine sulfate, must fall between $-.60°$ and $+.05°$.[362] To illustrate how the tropine moiety influences the specific rotation, d- and l-tropic acids have specific rotations of $+72.2°$ and $-72.2°$, respectively.[237a] Using standard conditions and solvents, specific rotation can aid in defining the purity of a particular isomer of hyoscyamine.[37] An investigator can make an initial identification for the periods of shelf-storage, and also check for mutorotation. Little information appears available regarding isomer stability, including mutorotation, for the hyoscyamines. Puech et al.[294] have demonstrated optical stability of d-hyoscyamine in accelerated tests and a shelf-life test of one year's duration. Further work should be undertaken to establish optical stability for the other isomers of hyoscyamine.

As esters, hyoscyamine isomers can hydrolyze in aqueous solution. Kondritzer and Zvirblis[193] showed that atropine hydrolysis reaches a minimum in aqueous solution between a pH of 3.24 and 4.11, depending upon the temperature. The pH range for atropine sulfate injection should fall between 3.0 and 6.5 according to the *United States Pharmacopeia,* 20th Edition.[362] Lu and Hummel[222] found that atropine lost approximately 13% of its labelled content upon standing for 2 years and 25% after 5 years, using a colorimetric test.[8] At a higher pH of 7, the rate of atropine hydrolysis amounted to 54% for 1 year at 25°C.[342] Biological activity declined to nearly the same levels as registered for the chemical assays[222] when ascertaining the degree of atropine hydrolysis. Storing of atropine solutions for long periods decreases their pharmacological activity by 35% after 5 years as determined by bioassay in several species.[148] Hydrolytic breakdown products of atropine did not produce any biological effect.[222]

XIV. PHARMACODYNAMICS AND MICROASSAYS FOR PLASMA LEVELS

Recent uses of atropine to combat serious bradyarrhythmias following myocardial infarcts and the continued interest in the use of atropine in combating the poisoning of individuals by organophosphate compounds demand a better understanding of the tissue disposition and elimination kinetics of atropine. Until relatively recently we have experienced a dearth of potentially sensitive techniques with good precision to measure small amounts, nanograms and picograms, of atropine/ml of blood. Early attempts to follow the fate of atropine relied upon ^{14}C labelled atropine.[122] With the development of pharmacokinetic models,[235] facilitated by computer programming, coupled with more sensitive separation and detection techniques, such as high performance liquid chromatography, radioimmunoassay, gas chromatography and mass spectrometry, we have reached a threshold whereby we can now study the fate of atropine, utilizing small blood samples from unsedated, unanesthetized animal models (humanely treated) and man using atraumatic procedures[181] already developed. Thus, we may soon have the potential to coordinate the cardiovascular responses of atropine with its pharmacokinetics. Further on in this section, a more detailed account of microassay procedures will appear. Initially, I would prefer focusing on recent studies that highlight the fate of atropine following its administration.

In the rat, Harrison et al.[138] found that after intraperitoneal injection of atropine, the greatest amount appeared in the kidney, followed by the liver, adipose tissue and heart in descending order of amounts. The elimination rate of atropine from the heart and kidney took place most rapidly with brain and adipose tissue release of atropine occurring at the slowest rate. The heart took up atropine in a dose-related manner (0.2-0.22% of total dose at peak levels) based on tritium levels.[138] Following an intravenous bolus injection of atropine (0.2 mg/kg), a peak blood level of 70 ng/ml of atropine appeared within 2 minutes; the heart rate in the conscious dog peaked at the same time.[42] The subsequent decay in heart rate followed fairly closely the steady decline in blood levels of atropine.[42] Likewise in man, Kalser and McLain[167] demonstrated a close relationship between the increase in radioactive atropine in the blood and the acceleration in heart rate following an intramuscular injection of atropine. The peak heart rate and atropine blood level following intramuscular injection of atropine materialized within 30 to 40 minutes in man.[167] As one might expect, the peak responses following an intravenous bolus of atropine declined more rapidly[42] than those following an intramuscular injection. However, the blood levels of atropine in the dog, declined more rapidly (estimated blood ½ time: 33 minutes) than the heart rate does (estimated ½ time: 70 minutes).[42] Following intramuscular administration of atropine in man, heart rate remained elevated for a considerable period of time, then subsides slowly.[42] In a more recent study in man (surgical patients), using a radioimmunoassay technique for evaluating plasma atropine levels, 2 minutes following an intravenous bolus injection of atropine (1 mg), the plasma atropine level rose to 230 ng/ml then fell precipitously to 9 ng/ml by 10 minutes and thereafter receded slowly to 3.5 ng/ml by 1 hour.[24] In another experiment within the same study, the authors[24] followed a 1 mg intramuscular injection of atropine that produced a peak plasma concentration of 3 ng/ml at 30 minutes, which slowly fell to about one-half that level by 4 hours. The plasma level plot following intramuscular atropine has roughly the same contour as the heart rate plot of Kalser and McLain[167] following intramuscularly administered atropine. Thus, further experiments may demonstrate a close relationship between the decline in plasma levels of atropine and the reduction in heart rate following either a bolus intravenous injection of atropine or an intramuscular injection. Chassaing et al.[42] demonstrated that a slow intravenous infusion of atropine sulfate (0.2 mg/kg/hr) promoted an initial, transient bradycardia which reverted to a rapidly accelerated and marked tachycardia by 10 minutes. In contradistinction with the two previously described methods of atropine administration, the produced tachycardia subsequently, gradually subsided over a 2 hour period while plasma levels of atropine slowly augmented. Much additional work must take place before we can fully understand the muscarinic antagonisms of atropine against the cardiovascular system, and the attendant plasma levels of atropine, and the relationship to the rate of exposure to atropine at the receptor sites, and subsequent physiological responses as influenced by the route, duration and rapidity of administration.

Atropine exhibits two elimination half-lives, as defined in the human[167, 338] and the dog.[6, 395] In the

human during the first half-life elimination (1.8 ± .2 SEM hours), 20 percent of the total excretion took place via the kidney with 70 percent of this amount in the form of a glucuronide conjugate.[167] The second elimination phase, also via the kidneys, had an elimination half-life of 25 ± 6 hours with 57-73% excreted as the unconjugated form.[167] Kalser and McLain[167] used radiolabelled atropine; hence, from their studies one cannot tell whether parent drug, metabolites or a combination thereof represented the elimination product. Beerman and associates[23] administered ^{3}H-atropine intravenously to man; they established an initial elimination half-life of 2.5 hours and a second, longer half-life of an unspecified duration. They[44] had accounted for 66.1% of the elimination in the urine after 6 days with only a 1.5% recovery in the feces. Further, Beerman's group[23] established by electrophoretic technique that within 6 hours of oral administration, 51-68% of the atropine had metabolized whereas 70-95% metabolized following intravenous administration. In studies on 2 men, no radioactivity in expired air appeared after an intramuscular injection of 2 mg of ^{14}C atropine.[22] There may be great variability in the rate of elimination of atropine from individual-to-individual, for one person eliminated 50 percent in 4 hours and 88 percent in 48 hours, whereas the other individual voided 18 percent of the radioactivity in 4 hours, yet excreted 85 percent via the urine in 24 hours.[122] Although they lacked proof, Gosselin and associates[122] felt that approximately 50 percent of the intramuscular dose of atropine appeared in the urine unmetabolized, whereas less than 0.5 percent showed up in pooled fecal samples. At 6 hours, Albanus et al.[6] accounted for 16.7 percent of the radioactivity in the gallbladder. In the dog, 30 percent of atropine excretion occurred via the urine in the unconjugated form and they retrieved 50 percent of the atropine from the urine by 6 hours. At 10 hours, the concentration in the cerebral spinal fluid exceeded plasma levels by a factor of 10; at 2 hours the plasma and cerebral spinal fluid concentrations were equal; however, by 6 hours cerebral spinal fluid concentrations of radioactivity nearly doubled that found in the plasma.[6] Elimination of lower doses of atropine appeared more rapid than for larger doses.[6] In the dog, one must administer a minimal subcutaneous dose of 0.5 mg/kg of atropine in order to obtain central effects — these effects occurred with a minimal plasma level of 0.1 μg/ml.[5]

Studies by Albanus et al.[6] and Chassaing et al.[42] furnished sufficient information, such as atropine dose, peak plasma levels and the weights of dogs, to allow the reader to compute the percent of potential atropine concentration at the peak level of distribution. Using data from 16 studies,[9] I estimated that the circulating blood volume of the dog represented approximately 9 percent of body weight (89.98 ± .07 SD ml/kg). I assumed total homogeneous distribution of atropine within the active circulating volume of the dog at peak response. Accepting these assumptions as such, the peak concentration of atropine in the Albanus study[6] amounted to 3.6 percent of the theoretical maximum, while in the Chassaing study,[42] I computed 3.8 percent of the maximum amount. Although these percentages represent a hypothetical fraction from a theoretical maximum, which probably never exists, I believe that the very similarly, exceedingly low percentages do represent and substantiate the observations of Harrison's group[138] that organ systems probably take up atropine from the blood at a very rapid rate, hence leaving only a small residual concentration within the blood plasma. Does this mean that the presence of peak response and the peak levels of Kalser and McLain[167] and high levels of Chassaing et al.,[42] represent observations of coincidence and not causal? Do these observations point to further studies which will demonstrate a relationship between organ tissue-blood levels of atropine and their uptake-loss balance and this relationship to the blood-kidney exchange with respect to atropine excretion via the urine? Certainly, as the pharmacokinetic field grows with its ever increasing techniques, resources and computer assisted evaluations more vistas will come to the fore and more questions will have answers with respect to the meager kinetic knowledge of atropine which we presently possess.

To date, our knowledge of atropine kinetics in cardiac tissue rests upon the *in vitro* study. Thron,[359] using an *in vitro* guinea pig atrium preparation, showed that atropine accelerated the periodicity of the tissue in a manner which represents nonlinear kinetics, thus ruling out simple diffusion of atropine as the rate-limiting step. Precisely, whether the reaction limitation comes about due to limiting the rate of atropine to the receptor site or to an atropine-receptor reaction could not be ascertained by Thron.[359] Until recently, the possibility for doing pharmacokinetic studies on muscarinic doses of atropine in animals and man appeared nil due to the low, initial dose (usually about 2 mg) and the subsequently low

plasma levels obtained to construct the necessary plots for an extended duration following atropine administration. In a review, Clark[44] outlined several techniques which, with additional refinement, may offer proper sensitivity and precision for atropine detection in the nanogram and picogram levels in plasma and urine. As of the writing of that review, gas liquid chromatography, high performance liquid chromatography, and the radioimmunoassay represented the leading techniques with the radioimmunoassay demonstrating the greatest sensitivity.

The radioimmunoassay can detect atropine in plasma in the 62.5 picogram to the 10 nanogram range.[24, 97, 395] Fasth et al.[97] assayed atropine in the presence of oximes. At the 15 ng/ml level, Berghem and coworkers[24] could recover 82.6 percent of the known amount of atropine with a precision of 4.4 percent, as defined by the coefficient of variation for 19 samples. The radioimmunoassay discriminates and has specificity for atropine as opposed to atropine hydrolytic breakdown products and acetylcholine.[395] However, metabolites, such as 4-hydroxyatropine or atropine glucuronides can interfere.[400] The assay protocol calls for the use of rabbit serum in the production of specific antibodies for atropine; the possibility always exists that an undetectable amount of atropine exists in rabbit serum.[395] Rabbit atropinase preferentially hydrolyzes the l-hyoscyamine.[97, 395] Possibly, due to rabbit atropinease, the antibody possesses more affinity for the d isomer. Of course, pharmacokinetic studies in animals having atropinease, thereby tending to store d-hyoscyamine, could produce falsely high atropine values in such animals. Berghem et al.[24] may have overcome the problem of d-hyoscyamine specificity as they used antiserum that recognized both hyoscyamine isomers; however, use of their technique might still lead to erroneous atropine level estimations in animals with atropinease. One important feature of the radioimmunoassay technique is the extremely low plasma volume samples required (50 μl).[395]

High performance liquid chromatography may not have sufficient sensitivity (15-150 μg) for the low levels of atropine in extended kinetic sampling.[377] The precision amounts to ± 1 percent and the technique does discriminate between hyoscyamine isomers.[352]

A relatively new method used in man depends upon the ability of atropine to compete with quinuclidinyl benzylate for muscarinic binding sites.[239] Sensitivity extends to the low picamol range with a 95 percent recovery or better at slightly higher concentrations. Further reported experiences should furnish a better definition for this technique's potential.

Bayne et al.[21] introduced a method for scopolamine determination, based upon earlier studies of Walle and Ehrsson,[380] using gas liquid chromatography and electron capture detection. This method detected scopolamine at the picogram level with good precision. Unfortunately, further studies using this method for microassay detection of atropine have not appeared in the literature. This method should work for the hyoscyamine alkaloids as well as for hyoscine. Previously, Blake and associates[29] used this method to assay low nanogram levels of cocaine. Evidently, this method has the potential for high sensitivity for many compounds, as evidenced by its use in determining pilocarpine in the picogram range.[22] Although the gas liquid chromatography-mass spectrometry method has not been utilized for atropine quantification at picogram levels, consideration for its use in determining atropine in plasma at these low levels would seem in order.

XV. SUMMARY AND CONCLUDING STATEMENTS

Whereas the vagotonic controls of heart rate decline in the presence of sufficient atropine which leads to the well-known cardioacceleration, many of the underlying actions of this drug upon the heart remain either controversial or unknown. In contrast with many of our pharmacological agents in use today, atropine represents a drug with a long history and thus most physicians and investigators have extensive experience using it as either a therapeutic agent or an experimental tool. The familiarity with the drug may rest more with its duration of use than with the extensive knowledge of its many areas of response within the heart. With a recent growing demand for reversing the threatening effects of organophosphate poisoning and postmyocardial infarct bradycardia of the heart, studies utilizing atropine as a muscarinic blocking agent have demonstrated our inadequate understanding of the effects of this drug upon the inotropy and chronotropy of the heart. This review primarily provides an overview of

atropine's actions upon the heart, addresses the known, unknown and equivocal bases for these observations, and relates the past experiences to present-day requirements.

The modern history of atropine covers a period of approximately 150 years. Observations attributing heart rate changes to atropine stem from the observations of Von Bezold and Bloebaum in 1867. Approximately 100 years ago, reports of isolation, synthesis and optical properties of atropine first appeared in the literature. Most of our current knowledge of the chemistry of atropine developed in the subsequent 30 years. The major advances in our current knowledge of pharmacologically active structural groups and those groups responsible for active isomerism and receptor function took place in the late 1950's and early 1960's.

A major impetus leading to the work which formed the nucleus for our present knowledge of atropine's influence upon heart rate took place as a result of Marris' observations, during World War I, that atropine did not cause a rapid increase in heart rate in patients with typhoid or paratyphoid fever. In the period following World War I, the agonist and antagonist actions of atropine upon heart rate became well-defined.

When animals or man exercised after taking atropine, exercise fatigue usually developed earlier and to a more severe degree than without the drug. Following an antagonist dose of atropine, the heart rate elevated; however, the heart would appear to perform at a less efficient level due to a reduction in stroke volume. Myocardial efficiency findings probably rest on tenuous grounds due to the use of nitrous oxide, which demands an extended steady state and fastidious technique to insure proper estimation of the partition coefficient for coronary blood flow determinations. Atropine causes an acceleration of heart rate, yet cardiac output appears to increase only moderately, if at all, due to the reduced stroke volume. Evidently, the reduced stroke volume occurs due to peripheral pooling, which reversed in humans following proper inflation of an antigravity suit. We do not know the mechanism responsible for this pooling effect. The reduced stroke volume observed in test subjects during exercise also appeared in humans undergoing baroreceptor tests involving positional changes after atropine.

The agonist properties of atropine, usually less than a systemic dose of 0.5 mg, which produces a reduced heart rate, have been attributed to its ability to accelerate acetylcholine synthesis within the central nervous system. Thus, the acetylcholine synthesized might stimulate efferent inhibitory parasympathetic impulses to the heart's pacemaker from the dorsal nucleus of the medulla oblongata. Other investigators attribute the agonist response either to a direct effect upon muscarinic receptors or possibly to a combined central nervous system receptor response.

In humans, an age-dependent response to atropine occurs, with the peak heart rate occurring in the 3rd decade of life, and a second, but lower level response takes place between the sixth and eighth decades. Evidence appears in the literature establishing that a tachyphylaxis can occur from repeated use of atropine, since diminished heart rates and recovery times appeared with subsequent doses. However, no controlled experiments have tested this hypothesis using heart rate as the affected response. A good correlation does exist between administered levels of atropine, peak heart rate response and peak blood levels of atropine.

In exercising men, those receiving atropine during an exercise test fared much poorer than those administered atropine prior to the exercise. In all those failing to complete the exercise test in a warm, mildly humid atmosphere, rectal temperatures registered higher and the sweat production appeared less. An adequate explanation for these observations has not been established; however, peripheral blood pooling caused by antagonism toward sympathetic controls of peripheral vascular smooth muscle tone or a direct relaxation of vascular smooth muscle could represent possible mechanisms. No doubt, the failure of sweat glands to function during an elevation in core temperature adds significantly to the observed exercise failure. We must await further studies to define the causes of exercise failure under various conditions of atropine administration and climatic conditions. Exercise and climate conditioning could ameliorate the severity of responses to exercise in humans receiving atropine.

In order to attain a thorough understanding of the muscarinic actions of atropine upon cardiac inotropy and chronotropy, we will require further functional and anatomical knowledge of the parasympathetic nervous system within the ventricular myocardium. Certain long-standing controversies require resolution. Most importantly, do the parasympathetic efferents innervate the ventricular contractile myocardium? If

so, do efferent parasympathetic stimuli promulgate a primary reduction in ventricular inotropy? In other words, do the changes of inotropy due to parasympathetic stimulation noted to date, represent secondary changes in response to a primary depression in heart rate—a representative mechanism illustrating a negative Bowditch effect? To date, histochemical studies and some electrophysiological studies indicate that a vast majority of the parasympathetic efferent innervation reaching the ventricles associates closely with the ventricular conduction system. Further, using histochemical techniques for identifying acetylcholinesterase, and at times choline acetyltransferase, several groups have identified efferent parasympathetic fibers associated with the SA node, atrial musculature, AV node and major conduction bundles of the ventricles. These techniques base their validity on the assumption that associated enzymes will always have a direct or contiguous association with the parasympathetic fibers. Efferent parasympathetic fibers did not come in contact with the contractile ventricular myocardiuim, nor did they innervate the peripheral Purkinje fiber network. Using anesthetized canine preparations, several groups have attributed a diminished ventricular contractility to direct parasympathetic stimulation of cardiac musculature. This contradistinction between the functional findings and anatomical observations requires resolution prior to our gaining full insight into the effects of atropine upon ventricular contractility and bundle branch conduction. The atrium does have acetylcholinesterase; therefore, one could propose a direct modulation of atrial contractility and conduction by atropine.

Much of our current knowledge about muscarinic influence upon cardiac conduction and its modifications by atropine stems from clinical studies on patients with various forms of heart disease. Atropine decreases the sinus cycle length, which reflects heart rate, and atrioventricular conduction time, yet does not alter the ratio relationship between sinus cycle length and atrioventricular conduction time within the individual. The ratio relationship of sinus cycle length to atrioventricular conduction time differs from individual-to-individual. Heart rate changes linearly respond to atropine in a dose related manner. Patients having the sick sinus syndrome, or sinus bradycardia due to disease, show a markedly depressed response to atropine. Atropine can reverse first degree or type I second degree heart blocks, but not a type II second degree heart block. The effects of atropine upon the third degree heart block appears unpredictable. Atropine probably exhibits very little influence upon His bundle conduction.

The bradyarrhythmias associated with myocardial infarcts respond to atropine, particularly, if treatment occurs within 8 hours of the onset of the attack. No adequate explanation exists for the latter observation. The etiology of the postmyocardial infarct bradyarrhythmia is not well understood; a higher incidence of reduced heart rate has occurred following posterior myocardial infarcts than after anterior myocardial infarcts. Such observations have suggested a profound vagotonic effect upon the atrioventricular conduction system, which lies in the posterior region of the heart. However, not all patients seem to respond in a uniform manner to the atropine, since some develop life threatening tachyarrhythmias. No doubt, myocardial irritability due to regional hypoxia after such attacks, could explain the observed tachyarrhythmias following atropine. An unresolved question of similarities of etiology for the bradycardia that develops in the trained athlete and in the postischemic insult exists, yet in both cases atropine seemingly can affect the same muscarinic responses. Atropine attenuates the bradyarrhythmias associated with exercise training and can accelerate the slow postmyocardial infarct rhythm. A major consideration in the evaluation of atropine upon myocardial arrhythmias rests with oxygen levels of the heart. In hypoxic conditions, atropine promotes a higher incidence of tachyarrhythmias, including ventricular fibrillation. Patients with occlusive coronary disease developed a greater incidence of autonomic disturbances following administration of atropine than those having a patent coronary circulation. As a general rule muscarinic agents increase the fibrillation threshold and atropine serves to nullify this response. The latter will probably only take place when the sympathetic nervous system to the heart remains active. Atropine in the presence of exercise causes a reduction in fibrillation threshold. Acetylcholinesterase inhibition, such as with organophosphates, elevates the fibrillation threshold and can cause atrial standstill, complete heart block, idioventricular rhythm and marked hypotension due to bradyarrhythmias. Using atropine to counter the organophosphate intoxicaiton of the heart may lead to fibrillation due to the attendant hypoxia of the heart. Recent studies indicate that muscarinic receptors on adrenergic fibers in the heart and peripheral circulation may function as throttles upon sympathetic tone. Atropine,

through its vagolytic action, may reduce the throttle ultimately augmenting sympathetic response. Possibly, acetylcholine functions to throttle sympathetic transmission by hyperpolarization. Our knowledge of the influence of atropine upon the atrial intranodal fibers remains incomplete and not well understood. Atropine attenuates the effective and functional recovery periods of the atrioventricular node but not the ventricular conduction bundles, and it does not appear to influence the effective atrial refractory period. At present, such statements do not stand unequivocally.

A brief discussion of intrinsic and extrinsic controls of the heart points to the necessary support of autonomics in other than basal conditions, and , particularly, to the preponderance of the parasympathetics. The autonomic systems function in a complex manner not in a straight algebraic manner, possibly through adenosine monophosphate and guanosine monophosphate.

Atropine represents a racemic mixture of d- and l-hyoscyamine. Hyoscyamine is an ester comprised of tropine and tropic acid. The point of active optical isomerism resides in the ß-carbon of the tropic acid. The methylpiperidine portion of the tropine, in the chair configuration, is the site of pharmacological action. Atropine sulfate monohydrate typifies the most readily used form of atropine. Generally, l-hyoscyamine possesses roughly 2 times the potency of atropine and approximately 30 times the activity of its optical isomer. Hyoscyamine isomers theoretically can change optically due to racemization or mutorotation. However, no evidence exists to establish that atropine mutorotates, or reverts back to d-hyoscyamine or l-hyoscyamine. Hyoscyamine isomers can hydrolyze to form tropine and tropic acid. Neither tropine nor tropic acid demonstrates appreciable biological activity. At a pH between 3.24 – 4.11 hyoscyamine isomers possess the greatest stability with minimal hydrolysis in solution. About 25% of the atropine in solution will hydrolyze in 5 years at room temperature, losing a comparable amount of biological activity. At a higher pH of 7, l-hyoscyamine, at 25°C, hydrolyzed 54% within 1 year. The USP standards constitute a broader range of pH (3.0 – 6.5) than is indicated above, thereby exposing the user to a potentially greater variability of potency.

Cardiac tissues readily take up atropine in a dose related manner and lose atropine in proportion to the blood levels; that is, as blood levels decline the heart levels recede at a similar rate. Peak heart rate takes place within 2 minutes of an intravenous injection of atropine, whereas a peak response occurred within 30 to 40 minutes following an intramuscular injection. A limiting factor in understanding the terminal elimination phase of atropine with respect to plasma levels and concomitant heart rate responses in pharmacokinetic studies involves the lack of a sensitive, reliable assay for atropine in the picogram-nanogram range. In recent years a radioimmunoassay technique has shown promise, however, presence of l-atropinease in the rabbit's serum has caused problems in linearity and recovery. High performance liquid chromatography may not have sufficient sensitivity. An assay matching atropine and quinuclidinyl benzoate as competitors for muscarinic binding sites may have sensitivity in the low picomol range. An assay method utilizing gas chromatography and electron capture has proven successful at the picogram levels for scopolamine, and should work for atropine; however, no published atropine studies have appeared as yet.

Conclusion: Our knowledge of the effects of atropine upon heart rate is extensive. The effects of atropine upon the heart during exertion and its metabolic costs require further studies. A body of literature concerning the effects of atropine upon the inotropy and chronotropy of the heart exists, yet much controversy remains. This controversy prevails due to our incomplete knowlege of the anatomic and functional relationships of the parasympathetic muscarinic system within the ventricular myocardium. In addition, we need to pay stricter attention to the stability of atropine, particularly when low doses are required.

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